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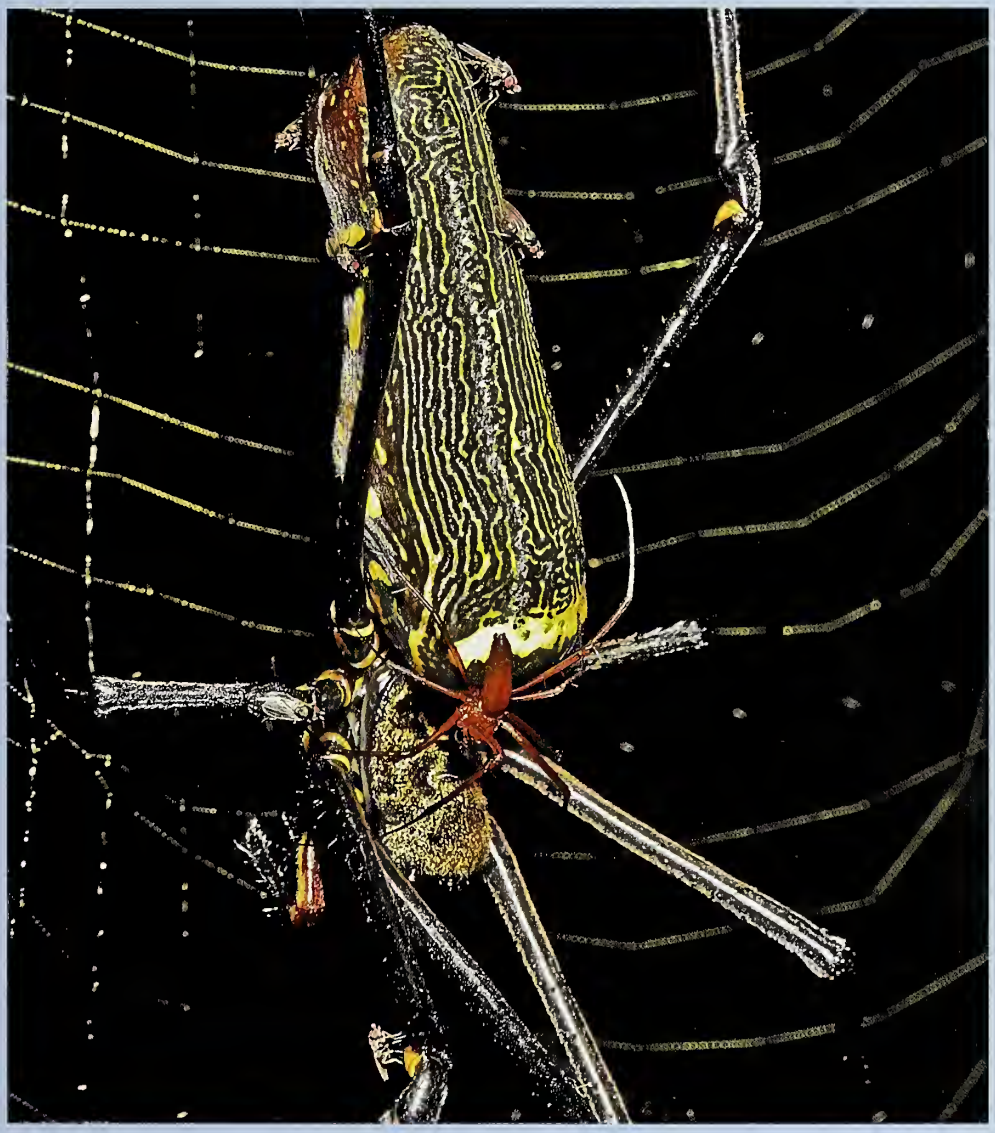

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Cover photo: Female giant wood spider, *Nephila pilipes* (Nephilidae), accompanied by a much smaller male and at least six kleptoparasitic flies, in Maylasia (see page 345). Photo by M. Kuntner.

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Distribution and morphology of the European Karst palpigrade *Eukoenenia gasparoi* (Arachnida: Palpigradi)

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Abstract. The first record of the palpigrade *Eukoenenia gasparoi* Condé 1988 in Croatia is reported. We review the distribution of this troglobiotic species endemic to the classical European Karst region, give an illustrated description of the adult stages and the juvenile female, and evaluate divergence among the populations. Morphological differences reflect individual-level rather than population-level variation.

Keywords: Cave, Croatia, endemism, first record, troglomorphy

Over 80% of the 28 palpigrade species recorded from Europe inhabit eaves and related subterranean habitats (Harvey 2003; Christian 2009). *Enkoenenia gasparoi* Condé 1988 belongs to a group of seven troglobiotic species characterized by at least three blades in the lateral organ and seven (exceptionally six or five) setae on the basitarsus of the last pair of legs, while the remaining European species have only one blade or four basitarsal setae. The group includes Europe's most distinctly modified cavernicolous palpigrades (Condé 1998) with consistently small distribution ranges: *E. brignolii* Condé 1979 from Apulia, Italy; *E. draco* (Peyerimhoff 1906) from Mallorca, with the subspecies *E. d. zariquieyi* (Condé 1951) from Catalonia, Spain; *E. grafittii* Condé & Heurtault 1994 from Sardinia, Italy; *E. hispanica* (Peyerimhoff 1908) from Aragon, Spain; *E. naxos* Condé 1989 from Irakleia, Greece; and *E. patrizii* (Condé 1956) from Sardinia, Italy. *Eukoenenia gasparoi* is currently the best documented member of this group and has been recorded from four localities. We report on habitat characteristics of a new location that widens the known distribution range of the species from the Classical European Karst landscapes in northeastern Italy and southwestern Slovenia toward the northwestern limits of Croatia. In order to detect possible intraspecific divergence, we supplement Condé's (1988, 1990) data with a detailed morphological description of the Croatian specimens.

METHODS

We fixed the three speeimens from the cave Radota jama in 70% EtOH, cleared them in 10% KOH and chloral phenol, and subsequently embedded them in a modification of the water soluble Swan medium (Rusek 1975). Next, we studied the slides under a Nikon E 600 mieroscope with a measuring eyepiece and phase contrast and DIC optics. Micrographs, taken with Nikon D 200 and Nikon 1 J1 cameras, were stacked and edited (contrast enhanced, motifs partly cut out) using Photoshop CS5.

Setae (in italics) are termed according to Condé (1988) except for the converse labeling of *gla* and *grt* (see footnote in Condé (1990:834). Abbreviations: B = length of propeltidium; ti = tibia; bta = basitarsus; ta = (telo)tarsus; a = maximum width of the basitarsal article (instead of the commonly used width at the insertion of seta *r*, because there the cross section of the basitarsus is not perfectly round: rotation of the article changes the apparent width); d*grt*, d*gla*, d*esp*, d*r*, d*esd* = shortest distance between the insertion of the seta and the base of the article; *fs* = forked seta(e). Indices (Table 1) are given as dividend/divisor.

The specimens are held in the eollection of the Croatian Biospeleological Society, Zagreb.

GENERAL DISTRIBUTION AND NEW RECORD OF *E. GASPAROI*

The known localities of *E. gasparoi* (Fig. 1) are situated in the western part of the Classieal European Karst, i.e., the Carso Triestino, the adjoining Low Littoral Karst, and its continuation in the karstic plateau of the Ćićarija. Although the five caves are situated along a NW–SE line no longer than 45 km, the range of distribution extends over borderlands of Italy, Slovenia, and Croatia. *Enkoenenia gasparoi* is the only endemic palpigrade species of a region where two cavernicolous congeners, *E. spelaea* (Peyerimhoff 1902) and *E. anstriaca* (Hansen 1926), have also been recorded (Zagmajster & Kováč 2006).

The locality of the first Croatian record, Radota jama (Figs. 2, 3), is an unbranched karst cave of 268 m length and 170 m depth, developed in dark brown miliolide limestone of the middle Paleocene. The entrance opens at 593 m a.s.l. on Mt. Žbevnica between the villages Rakitovec (Slovenia) and Brest (Croatia) on the Ćićarija plateau in the northern part of

Table 1.—*Eukoenenia gasparoi*, morphometric indices of all known specimens (leg 1 above, leg IV below the division; j = juvenile; see text for other abbreviations). New data are in bold, literature data are derived from Condé (1988, 1990). ♀1 = Grotta delle Perle, holotype; ♀2 = Grotta delle Perle, paratype; ♀3 = Grotta Azzurra di Samatorza; ♀4 = Vilenica jama; ♀5 = Radota jama; ♂1 = Vilenica jama; ♂2 = Radota jama; j♂1 = Grotta delle Perle; j♀1 = Caverna III del Monte Sedlen; j♀2 = Radota jama. * value for the opposite leg is 2.63.

	♀1	♀2	♀3	♀4	♀5	♂1	♂2	j♂1	j♀1	j♀2
bta3/*r*	1.00				**1.08**		**1.09**	0.98		**1.03**
bta3/d*r*	4.07	4.04	4.13	4.59	**4.74**	4.44	**4.32**	3.33		**4.05**
bta3/ti	0.38			0.37	**0.36**	0.37	**0.36**	0.36		**0.35**
bta3/B	0.24				**0.24**		**0.24**	0.20		**0.22**
bta/a	9.53				**8.89**		**8.89**	8.00		**8.45**
bta/*r*	2.80	2.80			**2.90**		**2.86**	2.53	2.50	**2.58**
bta/d*r*	2.42	2.42			**2.59**		**2.40**	3.44	3.15*	**3.15**
bta/ti	0.81			0.79	**0.81**	0.79	**0.77**	0.78		**0.82**
gla/grt	0.64	0.66	0.65	0.68	**0.77**	0.69	**0.71**			
bta/B	0.53	0.57	0.54		**0.55**		**0.55**	0.44	0.51	**0.51**

the Istrian peninsula, a few hundred meters within the territory of Croatia. Bat guano is a major nutrient basis for the rich cavernicolous fauna that includes the snail *Zospeum spelaeum schmidti* (Frauenfeld 1854) (Slapnik & Ozimec 2004), the spider *Mesostalita nocturna* (Roewer 1931), the pseudoscorpions *Chthonius spelaeophilus histrus* Beier 1931, *Troglochthonius doratodactylus* Helversen 1968 and *Neobisium reimoseri reimoseri* (Beier 1929), the woodlouse *Titanethes dahli* Verhoeff 1926 (Bedek et al. 2011), the centipede *Empolybothrus obrovensis* (Verhoeff 1930), the springtails *Absolonia gigantea* (Absolon 1901) and *Troglopedetes pallidus* Absolon 1907 (det. M. Lukić), the leptodirine beetle *Bathysciotes khevenhuelleri* (Miller 1852), and the pselaphine beetle *Machaerites kastavensis* Pavićević & Ozimec 2009. For the period between November 2010 and June 2011, permanently recording instruments yielded a mean air temperature of 9.1°C (8.4–9.5) and a mean relative humidity of 99.5% (97.1–100) in the cave.

Family Eukoeneniidae Petrunkevitch 1955
Genus *Eukoenenia* Börner 1901

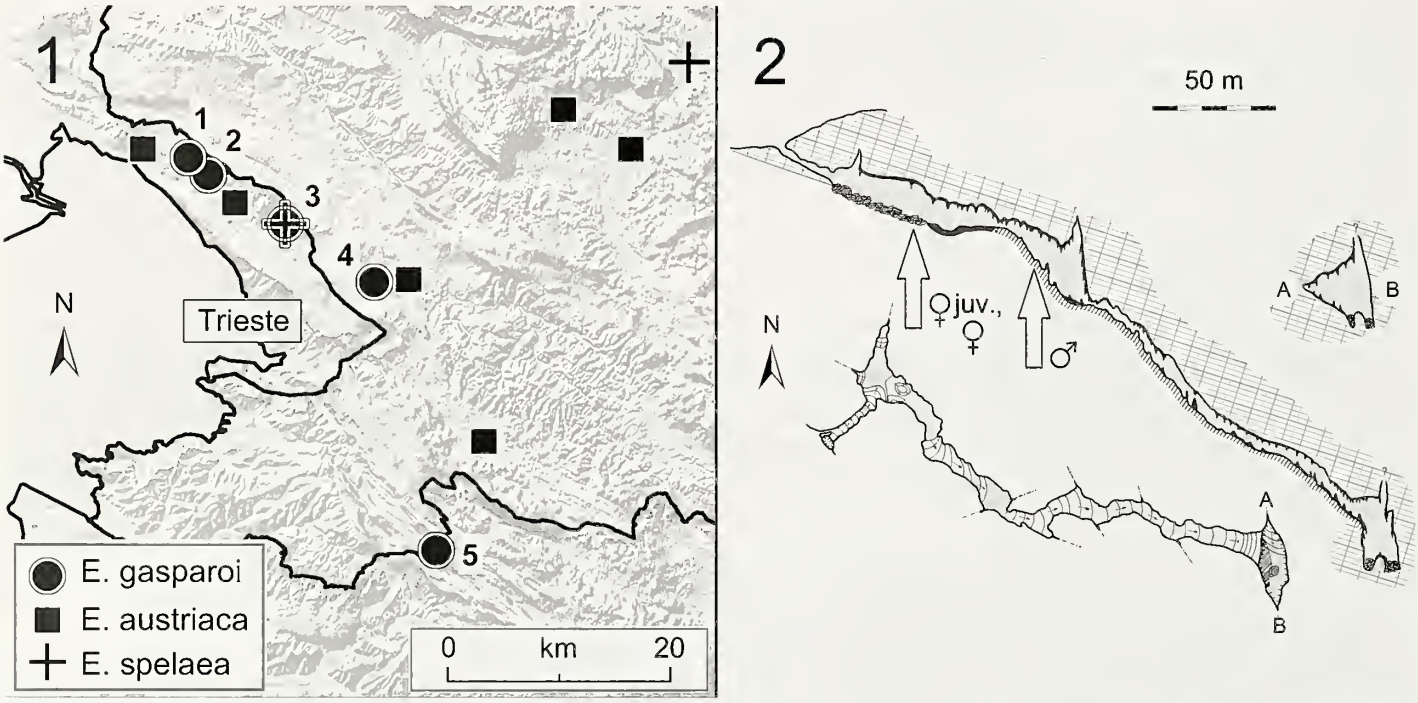
Type species.—*Koenenia mirabilis* Grassi and Calandruccio 1885, by monotypy.

Eukoenenia gasparoi Condé 1988

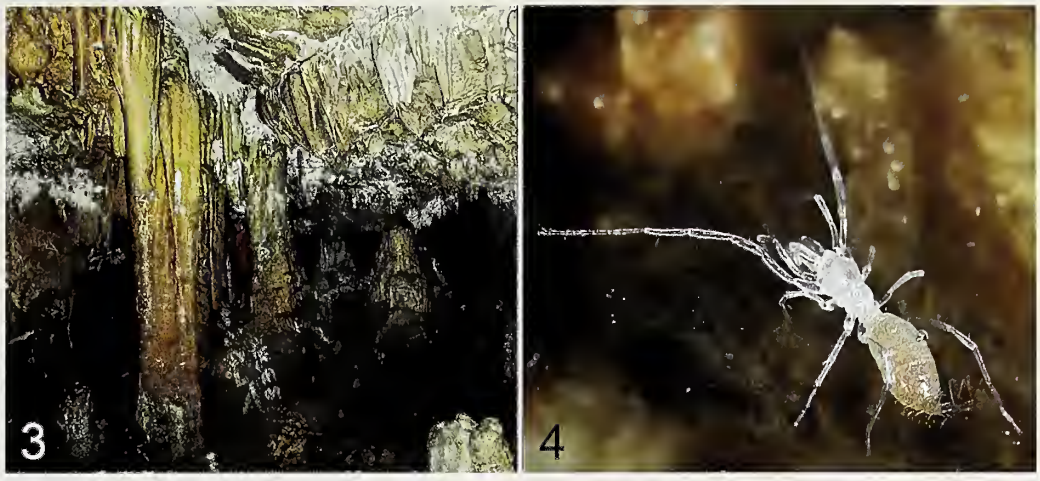
Eukoenenia gasparoi Condé 1988:729–736, Figs. 4–8, table 3; Condé 1990:833–835, Figs. 1c, 2c; Condé 1998:917, 919; Zagmajster & Kováč 2006:25, Fig. 1.

Material examined.—One adult male (leg. S. Polak) and one juvenile female (leg. V. Zakšek), collected from the underside of stones in the cave Radota jama on 27 November 2010 in the course of the Slovenian-Croatian Karst Underground Protection project (www.project-kup.org; Ozimec et al. 2011). One adult female (leg. R. Ozimec, 29 July 2001) from the same cave (Fig. 2).

Diagnosis.—A medium-sized, troglomorphic *Eukoenenia*. Adults with 3 blades in the lateral organ; 3 setal bases forming a wide V on the deuto-tritosternum; 10+10 very short setae on the propeltidium; 1+1 setae on the metapeltidium; 7 setae on the basitarsus of leg IV; tergites with only 1+1 setae *t*, flanked by one seta *s* on each side; sternites III–VI with 4+4 subequal setae; semiglobular bases of the fusules on the first genital valve of the



Figures 1,2.—Distribution and sampling maps. 1. Palpigrade records in the Classical Karst region. Overall distribution of *Eukoenenia gasparoi* as currently known: (1) Caverna III del Monte Sedlen (I); (2) Grotta Azzurra di Samatorza (I); (3) Grotta delle Perle (I); (4) Vilenica jama (SLO); (5) Radota jama (HR); data extracted from Condé (1988, 1990) and Zagmajster & Kováč (2006). 2. Radota jama (Croatia): section and plan view after Malez (1960), digitized and modified by D. Basara; arrows indicate the sampling locations of *Eukoenenia gasparoi*.



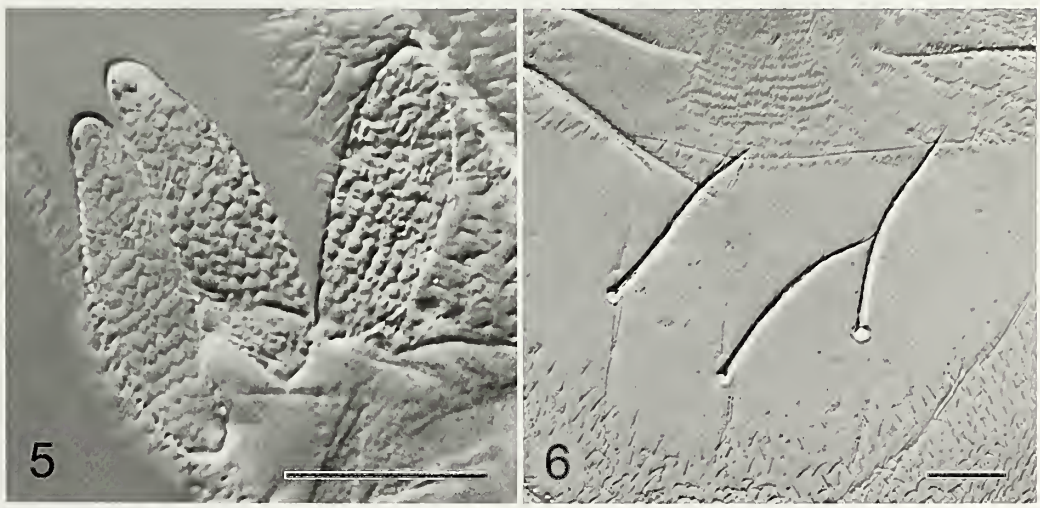
Figures 3,4.—Radota jama. 3. Habitat of *Eukoenenia gasparoi* (photo R. Ozimec). 4. *Eukoenenia gasparoi*, male, at the sampling spot in the Radota jama (photo S. Polak).

male, along with 4+4 and 5+5 setae on the following valves; 2+5+4 setae on each half of the first genital valve of the female.

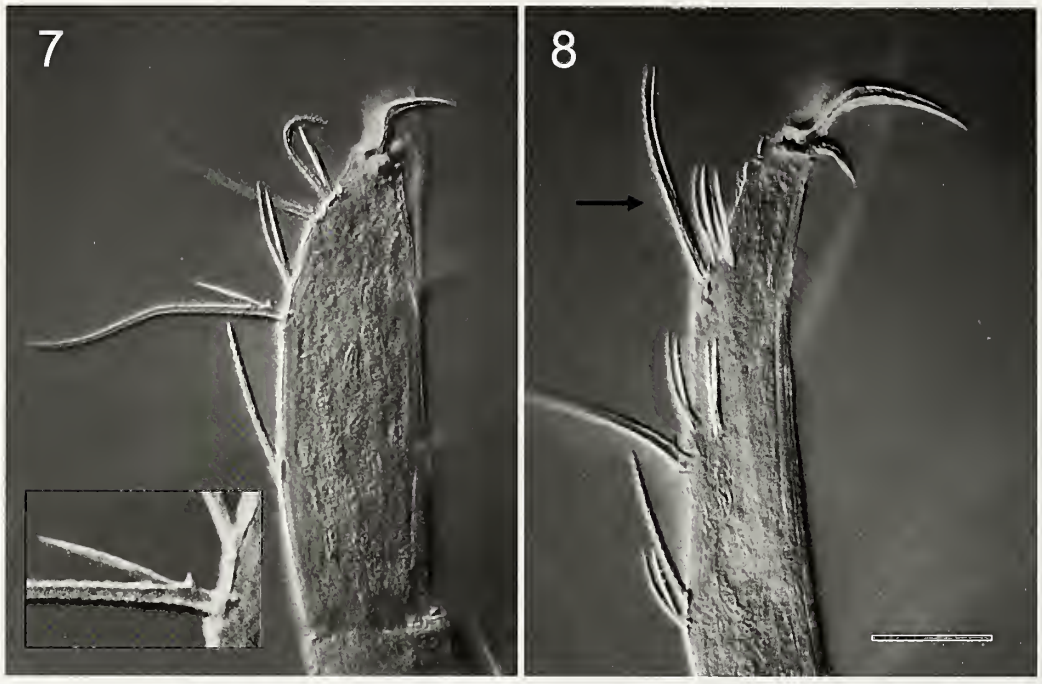
Description.—*Male:* Appearance as in Fig. 4. Body length (without the broken-off flagellum) 1560 µm. B = 440 µm. Pubescence mostly short and dense, somewhat longer and more sparse on the limbs, on opisthosomal segments IX–XI, and on labrum and deuto-tritosternum.

Frontal organ (crushed, hence overall length not determined) with a basal piece as long as the two broadly lanceolate branches, the latter abruptly narrowing to the rounded tip. The three elements of the lateral organ (37 µm) are relatively thick and evenly reticulated (Fig. 5). Propeltidium with 10+10 short setae (maximum 12 µm). Chaetotaxy of the metapeltidium reduced to 1+1 setae t_2 (76 µm). Labrum with the usual cuticular pattern and 5+5 setae (maximum 14 µm). Deuto-tritosternum with 3 subequal setae (maximum 57 µm) forming a widely obtuse angle (Fig. 6). Basal article of the chelicera with a proximal group of 6 setae (seta 4 and 6 thickened), in the distal half with 3 aligned setae (the last one longest), and 1 apical seta. Hand of the chelicera with 1 ventral and 6 dorsal setae. Fingers of the chelicera with 8 teeth.

Pedipalp in the proximal half of ta3 with a rodlike seta (23 µm) and 3 forked setae (*fs*) successively arranged in the distal half of the article (as in Fig. 7). The stiff smooth branches of the *fs* are about the same length (15–17 µm), the flexible barbed branch of the distal seta is moderately longer, that of the proximal seta considerably longer (48 µm). The proximal *fs* has, like other setae on the distal articles of the limbs, a denticle near its base. Length of pedipalp articles (µm): ti = 258, bta1 = 81, bta2 = 128, ta1 = 60, ta2 = 78, ta3 = 79. Leg I with 7 trichobothria at the usual positions and altogether 10 *fs*. The 6 *fs* of ta3 are arranged as in Fig. 8: 1 long + 2 short *fs* near the tip, followed by 1+1 and 1 short *fs* in equal distances towards the base of the article. The remaining 4 *fs* are inserted near the distal ends of ta2, bta4, bta2, and bta1; they are all long. Short *fs* (15–18 µm) are split almost down to the base; long *fs* (30–54 µm) are only furcate near the tip, but a longitudinal groove divides the shaft into a barbed and a smooth strand. The bta3 of leg I (Fig. 9) is over four times longer than wide and bears 1 short distal and 2 long setae: *grt* is a little longer than *r*, both are inserted within the proximal third of the article and surpass the base of bta4. On



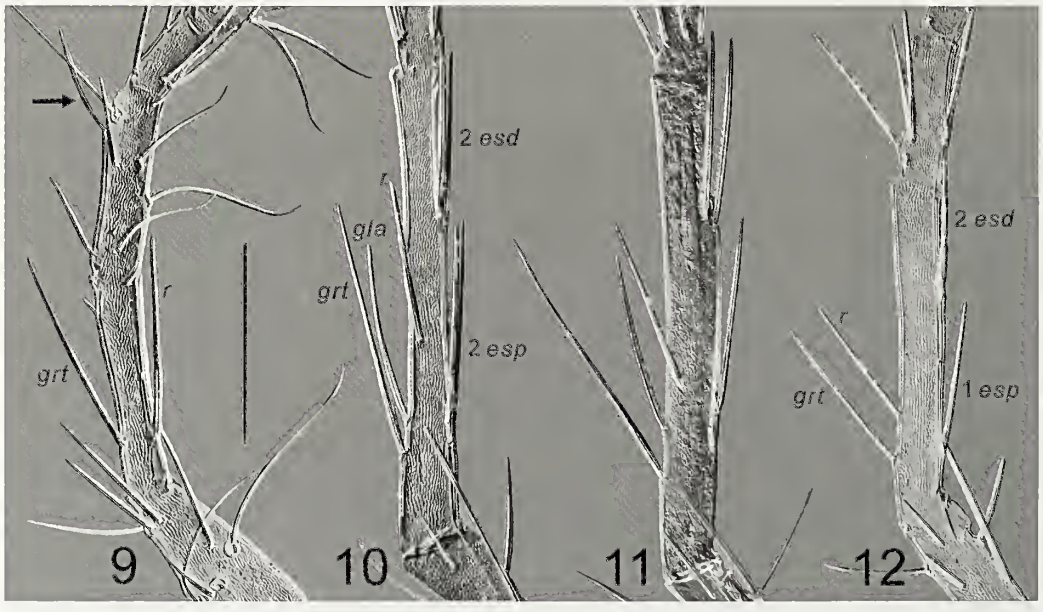
Figures 5,6.—*Eukoenenia gasparoi*, male from Radota jama: 5. Lateral organ; 6. Deuto-tritosternum. Scale = 20 µm.



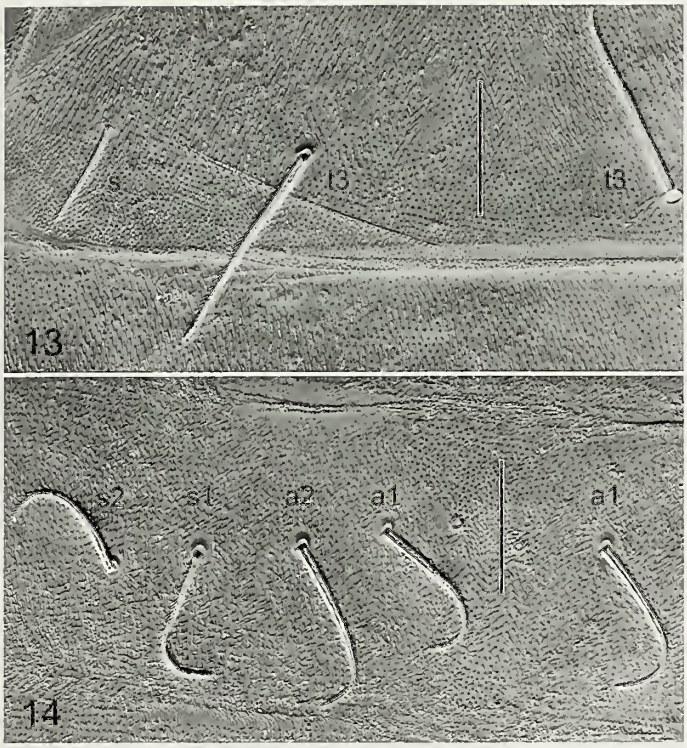
Figures 7,8.—*Eukoenenia gasparoi*, female from Radota jama, forked setae on terminal limb segments: 7. Pedipalp, ta3, with the base of the big forked seta enlarged to show the denticle ; 8. Leg I, distal half of ta3, with one long (arrow) and five short forked setae. Scale = 20 μm.

bta4, the trichobothrium, the *fs*, and 2 of the 5 ordinary setae are placed in the distal half. Measured values of leg I (μm): ti = 301, bta1+2 = 247, bta3 = 108, a = 26, *grt* = 104, d*grt* = 20, *r* = 99, d*r* = 25, bta4 = 91, ta1 = 43, ta2 = 47, ta3 = 193. Coxa of legs II and III each with 3 thickened setae (they are more cylindrical, apparently softer, and have an inclined barbule at the tip); rest of coxal chaetotaxy hardly readable. Leg IV with a very long and slender bta (nearly nine times longer than wide, Fig. 10), which bears 7 setae: *grt*, 2 *esp*, *gla*, *r* and 2 *esd*. Seta *r* is shortest, *grt* longest; all but *gla* carry a minute, oblique barbule at the tip. Measured values of leg IV (μm): ti = 310, bta = 240, a = 27, *grt* = 127, d*grt* = 50, *esp* = 109/96, d*esp* = 43/59, *gla* = 90, d*gla* = 67, *r* = 84, d*r* = 100, *esd* = 87/89, d*esd* = 152/158, ta1 = 84, ta2 = 96. Indices of leg I: bta3/a = 4.15, bta3/*r* = 1.09, bta3/d*r* = 4.32, bta3/ti = 0.36, bta3/B = 0.24. Indices of leg IV: bta/a = 8.89, bta/*r* = 2.86, bta/d*r* = 2.40, bta/ti = 0.77, bta/B = 0.55.

The opisthosoma shows a sudden narrowing between segments VIII and IX. Tergites II–VI with one seta *t* (probably t_3) and one seta *s*. Tergal setae *s* are considerably shorter than *t*, more slender and lack a typical basal ring (Fig. 13). Sternite III with st_2 and st_3. Sternites III–VI each with a pair of sternal



Figures 9–12.—*Eukoenenia gasparoi* from Radota jama: 9. Male, leg I, basitarsus 3 and 4, the arrow points to the forked seta next to the trichobothrium of basitarsus 4; 10. Male, leg IV, basitarsus; 11. Female, leg IV, basitarsus; 12. Juvenile female, leg IV, basitarsus. Scale = 100 μm.



Figures 13,14.—*Eukoenenia gasparoi*, male from Radota jama, opisthosomal chaetotaxy: 13. Tergite III; 14. Sternite IV. Scale = plane of symmetry = 50 μm.

pores and the setae a_1, a_2, s_1 and s_2 (Fig. 14), in the terminology of Condé (1988). The four setae of each hemisternum are of similar length, identical structure, and inserted at equal distances, so that they might as well be interpreted as a_{1-4}. On sternite VI, the setae a_1 are 66 μm long and 77 μm apart. Segments VII–XI with 7, 9, 8, 8, and 8 (maximum 101 μm) setae. The flagellum is lost.

Shape of the first genital lobe (Fig. 15) uncertain due to folding. It probably conforms to Fig. 8 in Condé (1988), just as the setation does. 2+2 sternal setae (st_1, st_2) are followed, on the lobe in the proper sense, by 11+11 phaneres including 2+2 fusules. Each fusule is inserted on top of a glabrous hemisphere through which the efferent duct shines (Fig. 16). The two flaps of the second lobe each with 4, those of the third lobe each with 5 setae (Fig. 15).

Female: Complementary observations (characters conform with those of the male partly omitted). Body length (without the broken-off flagellum) 1590 μm. B = 450 μm. Blades of the lateral organ maximum 35 μm. Seta t_2 on the metapeltidium 89 μm. Deuto-tritosternal setae maximum 58 μm. Pedipalp on ta3 with a rodlike seta and 3 *fs* (Fig. 7) arranged and shaped just as in the male. Length of pedipalp articles (μm): ti = 252, bta1 = 97, bta2 = 129, ta1 = 59, ta2 = 79, ta3 = 84. Coxa of the pedipalp with 19 setae, coxae of legs I–IV with 15 (0 thickened) / 14 (3 thickened; Fig. 17) / 12 (3 thickened) / 8 (0 thickened) setae. Leg I with trichobothria and *fs* as in the male (Fig. 8), shape and chaetotaxy of bta3 and bta4 the same. Measured values of leg I (μm): ti = 301, bta1+2 = 247, bta3 = 109, a = 27, *grt* = 108, d*grt* = 18, *r* = 101, d*r* = 23, bta4 = 98, ta1 = 48, ta2 = 53, ta3 = 199. The proportions of the basitarsus of leg IV are almost the same as in the male, but the insertions of the setae (particularly *gla* and *r*) differ (Fig. 11). Measured values of leg IV (μm): ti = 309, bta = 249, a = 28, *grt* = 126, d*grt* = 59, *esp* = 106/107, d*esp* = 65/74, *gla* = 97, d*gla* = 76, *r* = 86, d*r* = 96, *esd* = 91/95, d*esd* = 172/174, ta1 = 82, ta2 = 96. Indices of leg I: bta3/a = 4.04, bta3/*r* = 1.08, bta3/d*r* = 4.74, bta3/ti = 0.36, bta3/B = 0.24. Indices of leg IV: bta/a = 8.89, bta/*r* = 2.90, bta/d*r* = 2.59, bta/ti = 0.81, bta/B = 0.55. Setation of the opisthosomal segments II–VI as in the male. On sternite VI the setae a_1 are 73 μm long and 64 μm apart. Segments VII–XI with 7, 9, 8, 8, and 8 (maximum 120 μm) setae.

First genital lobe with 22 setae, each half has 2 basal + 5 + 4 apical. The basal setae are longest (maximum 75 μm), the apical seta a_4, inserted somewhat above a_{1-3}, is 35 μm long (Fig. 18). The two flaps of the second genital lobe each with 3 setae.

Juvenile female: Body length (without the broken-off flagellum) 1440 μm. B = 365 μm. Frontal organ (39 μm) shaped as in the adult ♂. Lateral organ (Fig. 19) with two blades (31 μm). Labrum with 4+4 setae. Deuto-tritosternum (Fig. 20) with 1 seta (47 μm). Chelicera with complete setation, fingers with 7 teeth.

Length of pedipalp articles (μm): ti = 193, bta1 = 68, bta2 = 105, ta1 = 53, ta2 = 69, ta3 = 71. Propeltidium with 11+11 short setae (i.e., with one pair more than the adult ♂). Metapeltidium with 1+1 setae (74 μm). Legs with trichobothria, *fs*, and thickened coxal setae as in the adult. Measured values of leg I (μm): ti = 230, bta1+2 = 171, bta3 = 81, a = 24, *grt* = 81, d*grt* = 11, *r* = 79, d*r* = 20, bta4 = 76, ta1 = 42, ta2 = 42, ta3 = 170. Basitarsus of leg IV (Fig. 12) with incomplete setation (*gla* and 1 *esp* missing). Measured values of leg IV (μm): ti = 227, bta = 186, a = 22, *grt* = 89, d*grt* = 35, *esp* = 82, d*esp* = 26, *r* = 72, d*r* = 59, *esd* = 77/74, d*esd* = 111/110, ta1 = 74, ta2 = 86. Indices of leg I: bta3/a = 3.38, bta3/*r* = 1.03, bta3/d*r* = 4.05, bta3/ti = 0.35, bta3/B = 0.22. Indices of leg IV: bta/a = 8.45, bta/*r* = 2.58, bta/d*r* = 3.15, bta/ti = 0.82, bta/B = 0.51.

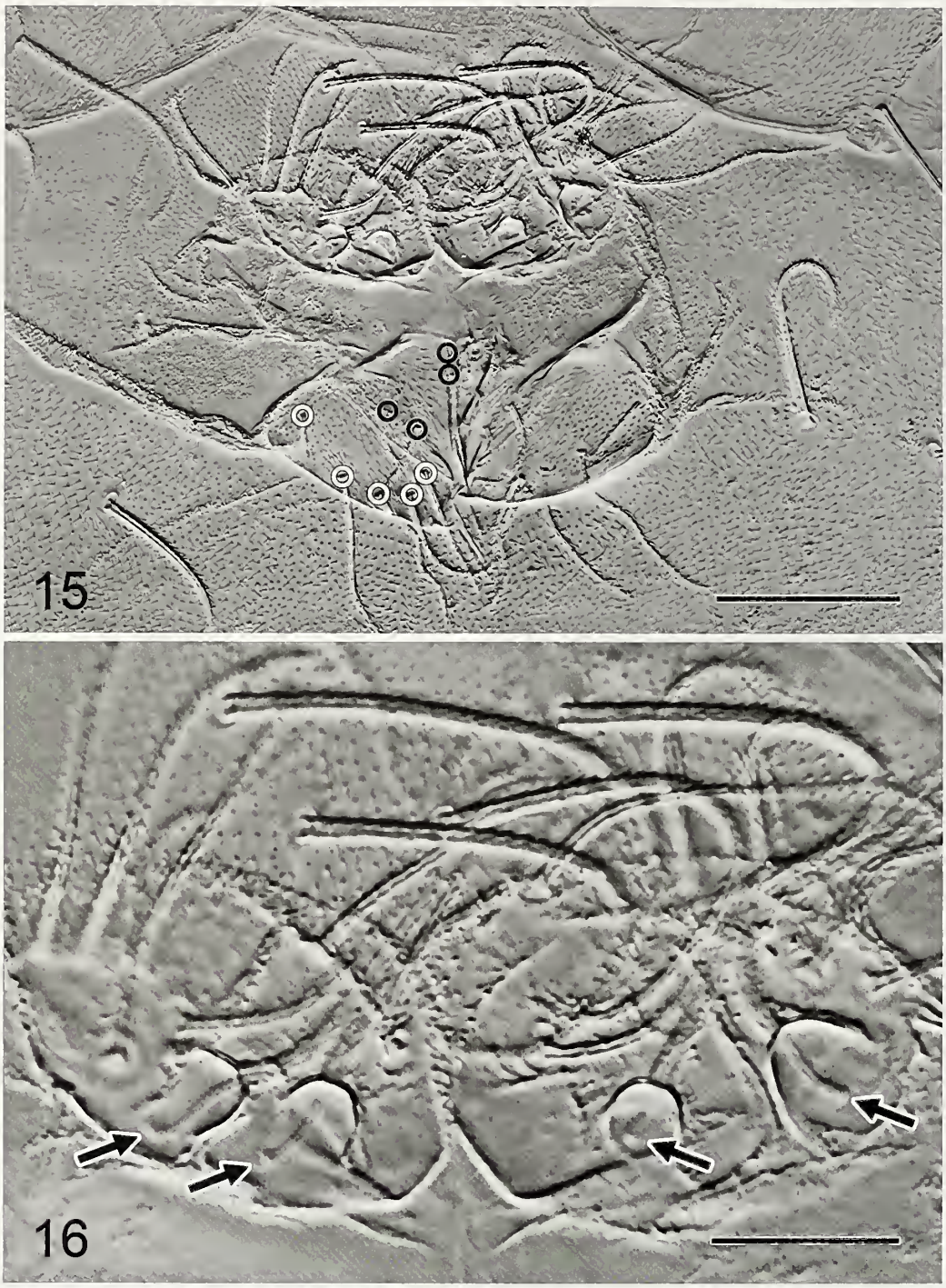
Primordia of genital valves are developed on opisthosomal segments II and III (Fig. 21). On the unpaired, but mediodistally cleft anterior valve of the juvenile ♀ 4+4 long and 2+1 minute subapical setae; the longest, most anterior pair (51 μm) probably represents 1+1 st_1. The posterior valve is composed of two lobes, each with 1 short seta. Two long setae at either side of the posterior valve are the sternal setae of segment III, st_2 and st_3.

Chaetotaxy of tergites II–VI as in the adult. Sternites IV–VI with a_1, a_2, and s_1; setae s_2 are missing (Fig. 22). On sternite VI, the setae a_1 are 61 μm long and 79 μm apart. All gland orifices are visible. Segment VII with 7, XI with 8 setae (chaetotaxy of VIII–X unclear).

Variability.—Table 1 compiles all available indices of the presently known specimens. The values lie closely together, suggesting that there has been virtually no morphological divergence among the Italian, Slovenian, and Croatian populations of *E. gasparoi*.

DISCUSSION

Cavernicolous palpigrades differ in morphological characteristics thought to be influenced by the subterranean environment; some species appear more troglomorphic, while others appear less so (sensu Christiansen 1962). Although



Figures 15,16.—*Eukoenenia gasparoi*, male from Radota jama: 15. Total aspect of the genital valves; black rings indicate setal bases of the second valve, white rings those of the third valve. Scale = 50 μm; 16. First genital valve. Arrows point at the hemispheric bases of the fusules. Scale = 20 μm.

Eukoenenia gasparoi has a moderately long flagellum of 1.30 times the body length (Condé 1990) and a common number of blades in the lateral organ, the elongated ambulatory and sensory limbs immediately suggest troglomorphy. This impression is supported by indicator indices such as the basitarsus IV proportion. Condé (1998) reports length/width values between 3.22 and 10.22 for cave-dwelling palpigrades, with *E. gasparoi* (9.53, holotype) ranking among the most troglomorphic species. In specimens from Radota jama, this ratio is 8.89 (♂ and ♀) and 8.45 (juv. ♀). According to Condé (1996), the length ratio basitarsus IV/propeltidium separates soil from cave palpigrades, averaging 0.28 in the former and 0.59 in the latter. Again the Croatian specimens turn out to be troglomorphic: 0.55 (♂ and ♀) and 0.51 (juv. ♀). The two indices suggest increasing troglomorphism from the juvenile to the adult stage, and so does the length of the foreleg. It is about 1.30 the body length in the adult specimens from Radota jama, but just 1.07 in the juvenile female.

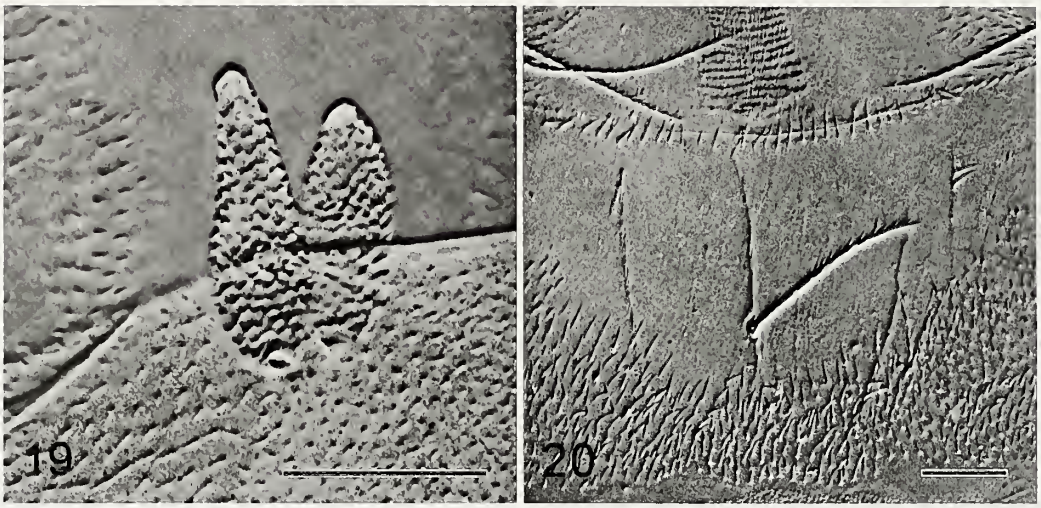
Figure 17.—*Eukoenenia gasparoi*, female from Radota jama, coxa of leg II; circles indicate the insertions of the thickened setae. Scale = 50 μm.

Figure 18.—*Eukoenenia gasparoi*, female from Radota jama, first genital valve; the dotted line indicates the plane of symmetry. Scale = 20 μm.

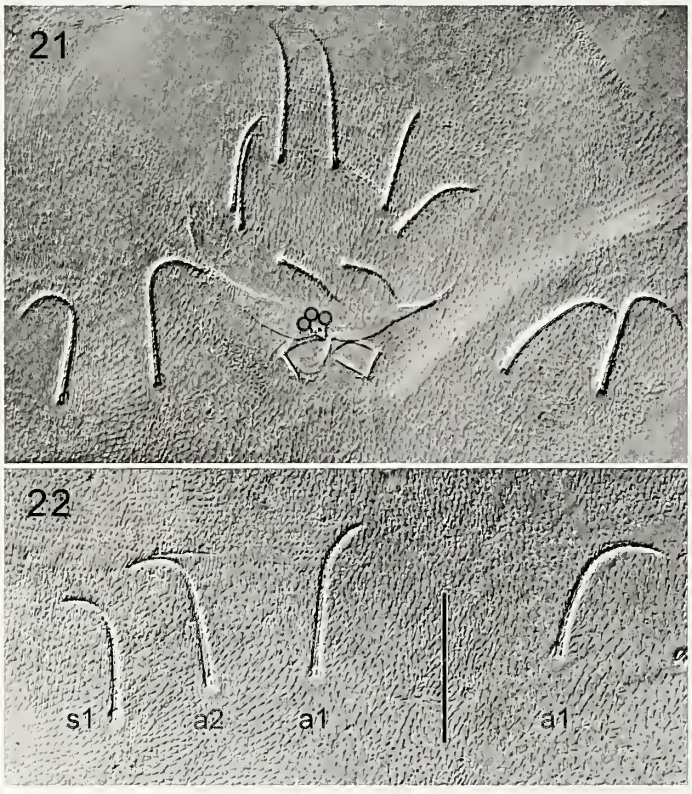
The length of basitarsus IV in relation to the tibia has also been considered informative for the degree of cave adaptation. Condé (1990) established bta/ti values of 0.70–1.00 for most of the cavernicolous palpigrades and 1.10 for the extremely troglomorphic *E. naxos*. Souza & Ferreira (2010) determined a bta/ti value of 1.07 for *E. maquinensis* from Brazil, a troglobiotic species with an excessively long flagellum. In *E. gasparoi*, however, the basitarsus of leg IV is clearly shorter than the tibia (bta/ti between and 0.77 and 0.82), suggesting moderate troglomorphy. The apparent inconsistency between bta/B and bta/ti leads us to conclude that any index, taken individually, may be an unreliable indicator of palpigrade troglomorphy. A long basitarsus may be caused either by an elongation of this very structure, or of the entire leg, or both. Furthermore, the comparison among related species shows that morphological changes in response to subterranean life do not necessarily evolve at the same pace, if they evolve in parallel at all. Many troglobiotic animals, cave fishes included, display a mosaic of more or less troglomorphic traits (Romero 2011). In *E. naxos*, for instance, all relevant characters indicate "most advanced subterranean evolution", except the number of blades in the lateral organ (Condé 1990).

The vast majority of the 27 native European plus one introduced palpigrade species occur in caves (Christian 2009), but only six resemble *E. gasparoi* in regard to btaIV chaetotaxy. None of them has more than two setae *t* on the tergites III–VI, and two species (*E. naxos*, *E. patrizii*) share with *E. gasparoi* the reduction to a single seta *t*. Number, distribution, and shape of the forked setae on the distal articles of pedipalp and leg I separate *E. gasparoi* from the species of the *mirabilis*, *spelaea,* and *subangusta* complexes, but it remains unclear whether this is a shared character state of all the seven species with an elevated number of setae on btaIV. These definitely troglobiotic palpigrades, supposed descendants from an ancient tropical fauna (Condé 1998), are probably endemic to closely confined areas. Some have been recorded solely from the type locality, but considering the mostly accidental records of palpigrades in caves, one need not necessarily presume spot endemism. *Eukoenenia gasparoi* surpasses related species in the density of records, so that the area of distribution is taking shape. The record reported here expands the known range from the Classical European Karst in the hinterland of Trieste (Italy) and Divača (Slovenia) some 30 km to the southeast, by a small distance into Croatian territory. Two other, closely related palpigrade species, *E. spelaea* and *E. austriaca*, partly overlap the range of *E. gasparoi*, but have much wider distributions. *Eukoenenia gasparoi*, however, is endemic to the area traditionally called "the Karst"; it is the Karst palpigrade par excellence. Intensified biospeleological research might uncover occurrences of *E. gasparoi* in other caves of the Ćićarija and the heartland of Istria, but the species is obviously missing in the well-investigated Postojna and Kočevje regions. We do not know of any current threat to *E. gasparoi*, but the small area of distribution makes the species appear vulnerable. The maintainance of the populations lies in the responsibility of the three countries.

The specimens from Radota jama consolidate the key characteristics of *E. gasparoi* and make adults easily determined. Inspection of the deuto-tritosternum alone would be sufficient for identification, as the reduced chaetotaxy (three setal bases forming a wide V in adult specimens) is unique among European palpigrades. Consideration of additional



Figures 19,20.—*Eukoenenia gasparoi*, juvenile female from Radota jama: 19. Lateral organ; 20. Deuto-tritosternum. Scale = 20 μm.



Figures 21,22.—*Eukoenenia gasparoi*, juvenile female from Radota jama: 21. Genital valves, black rings indicate the bases of the minute distal setae of the first valve; 22. Chaetotaxy of the opisthosomal sternite IV. Scale = plane of symmetry = 50 μm.

characters (see Diagnosis) resolves all doubt about species affiliation.

Eukoenenia gasparoi appears to be morphologically uniform. Although existing descriptions (Condé 1988, 1990) give no information on the propeltidial chaetotaxy of juvenile specimens, we assume that the additional pair of setae on the propeltidium of the juvenile female from Radota jama is due to individual variation. The same applies to the unstable insertion height of certain setae of basitarsus IV (compare Figs. 10 and 11). As even the left and the right basitarsus of one individual can differ (e.g., asterisk in Table 1), we follow Condé (1988, 1990) in regarding this variation as taxonomically irrelevant.

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A new cave-dwelling species of *Spelaeobochica* (Pseudoscorpiones: Bochicidae) from Brazil

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Abstract. *Spelaeobochica iuiu* sp. n. is described from Lapa do Baixão limestone cave in the municipality of Iuiu (Bahia, Brazil). It is easily distinguished from the two other species of the genus, *S. allodentatus* Mahnert 2001 and *S. muchmorei* Andrade & Mahnert 2003, by its pedipalpal measurements and proportions, but particularly by the presence of tooth-like protuberances on the pedipalpal segments. It is considered a troglobitic species.

Keywords: Karst, morphology, Neotropics, troglobitic

In Brazil, karstic areas are economically explored by several mining companies; as a consequence, many caves have disappeared with their fauna unknown or poorly studied. Different forms of anthropogenic activities such as quarrying, agriculture, waste disposal and tourism also take place in different parts of the country, which are liable to significantly affect cave communities (Ferreira & Martins 2001). Recent research documenting the cave biota of Brazil has intensified with the goal of protecting these endangered biotopes and their fauna (Souza-Silva et al. 2011).

Existing knowledge of the cave-dwelling pseudoscorpions from Brazil was summarized by Mahnert (2001), who recorded 25 species in seven families from about 100 caves, including the new genus *Spelaeobochica* and the type species *S. allodentatus* Mahnert 2001 from Bahia State. Subsequently, a second species of *Spelaeobochica*, *S. muchmorei* Andrade & Mahnert 2003, was added by Andrade & Mahnert (2003) from caves located in São Paulo State, Brazil.

The family Bochicidae ranges from Texas and Mexico to South America, from the Antilles to Venezuela, Guyana and Brazil, as well as in Europe (Spain, Portugal) (Muchmore 1998; Mahnert 2001; Zaragoza 2004; Reboleira et al. 2010).

The discovery of this new species in Bahia represents the third species of the genus *Spelaeobochica*. It inhabits the Lapa do Baixão cave (limestone karstic area), and it is highly adapted to the hypogean environment.

METHODS

Field work was performed in Lapa do Baixão cave (14°23′8.13″S, 43°37′35.06″W). The pseudoscorpions were found by visual searching of the cave floor and walls, captured with a fine brush, and placed in vials with 70% ethanol. We examined the specimens used for morphological analysis as temporary glycerine mounts in cavity slides. After examination, specimens were returned to 70% ethanol in glass vials. Measurements and drawings were made using a drawing tube with a phase contrast microscope. We studied one specimen (ISLA–846) using a scanning electron microscope. Parts of the female paratype were assembled on aluminum support stubs, placed over a film of aluminum foil with carbon tape, sputter-covered with gold (Baltec SCD 050), and observed in a LEO EVO 40 XVP scanning electron microscope (Leo Electron Microscopy). The ratios given are the length/width and length/depth for the legs. When two articles are compared, the ratio is the length/length index, and all measurements are listed in millimeters. Terminology follows Chamberlin (1931), Harvey (1992) and Judson (2007).

The specimens are lodged in the collection of invertebrates in the Laboratory of Subterranean Ecology, Biology Department of Lavras Federal University, Minas Gerais State, Brazil (ISLA); a paratype male (ISLA845) is deposited in the Museum of Natural History of the city of Geneva (MHNG).

TAXONOMY

Family Bochicidae Chamberlin 1930
Genus *Spelaeobochica* Mahnert 2001

Type species.—*Spelaeobochica allodentatus* Mahnert 2001, by original designation.

Spelaeobochica iuiu new species
(Figs. 1–19)

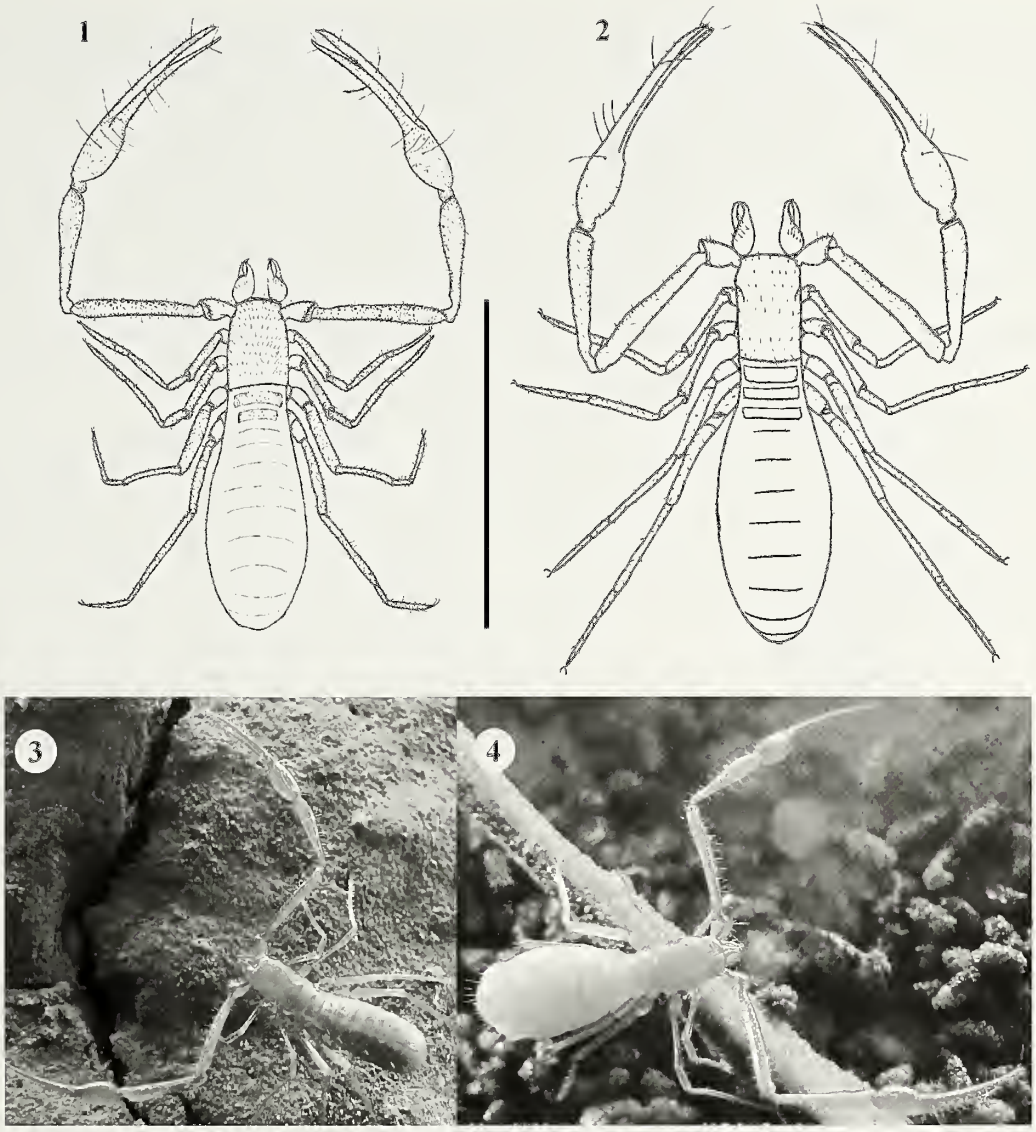
Material examined.—BRAZIL: *Bahia*: holotype female (slightly damaged, distal third of the left palpal finger missing), Lapa do Baixão cave, Iuiu (14°23′8.13″S, 43°37′35.06″W), 8 June 2010, R.L. Ferreira (ISLA–844). Paratypes: 2 males, same locality, 8 June 2010, R.L. Ferreira (1 each ISLA–846; MHNG–845); 1 female, same locality, 8 June 2010, (ISLA–842); 1 tritonymph, 20 July 2007, R.L. Ferreira (ISLA–843).

Etymology.—The specific name *iuiu* is treated as a noun in apposition; in Portuguese, it refers to the name of the municipality that the specimen inhabits.

Diagnosis.—The new species is characterized by the slender pedipalps (femur 7.8–8.3 times, patella 5.6–6.6 times, chela 6.1–6.9 times as long as broad), by the finely granulate pedipalps and, particularly, by the presence of tooth-like protuberances on femur and patella, and the presence of one tooth-like protuberance on medial side of chelal hand near the base of the fixed finger.

Description of adults.—Pedipalps, carapace, chelicerae, first coxae, and first abdominal segments reddish brown; other parts of the body yellowish brown. Tergites and sternites with light sclerotization along anterior margin. Vestitural setae smooth, delicate, and long.

Carapace: 1.6 times longer than broad, broadest near middle, then slightly narrowed to base, anterior margin smoothly rounded, without epistome, a broad reticulate transverse band near posterior margin; without eyes; 60 setae



Figures 1–4.—*Spelaeobochica iuiu* new species: 1. Habitus of male paratype; 2. Habitus of female holotype (scale bar 4.5 mm); 3, 4. Photographs of living specimens.

(6 setae on anterior margin and 6 on posterior margin), the range for paratypes is 50–60 setae.

Chelicera (Figs. 5, 15, 16): with 10 (11 on ♂) dorsal and 3 lateroventral setae on hand, fixed finger with 9 small teeth, (♂: 8–6), movable finger with 5 acute teeth, (♀ and ♂: 4) and a large, laterally displaced subterminal tooth, which is continuous with the remaining teeth; galea simple, slender and acute; subgaleal seta reaching end of galea; serrula exterior with 27 blades (♀: 28, ♂: 27–30), serrula interior with 23 blades (♀: 18, ♂: 22–23); rallum (Fig. 6) of 4 apically dentate blades, the two distal blades closely set.

Tergites: undivided, chaetotaxy: 6: 6: 6: 6: 9: 10: 9: 9: 9: 7: 6 [♂: 6: 5–6: 6–7: 6–8: 8–9: 8–10: 8–9: 9–10: 9–11: 7: 6–7; ♀: 6: 4: 6: 8: 8: 9: 11: 12: 7: 6]. Pleural membranes smoothly, longitudinally striate; manducatory process acute, with 2 apical marginal and 1–2 discal setae, 1 short (sub-oral) seta on palpal coxa; palpal coxa laterally scaly and sculptured, with numerous tiny pores, 6–8 long setae, coxae with numerous tiny pores (number decreasing from I to IV), I with 4–5 setae, II 4–5, III 4, IV 4–6.

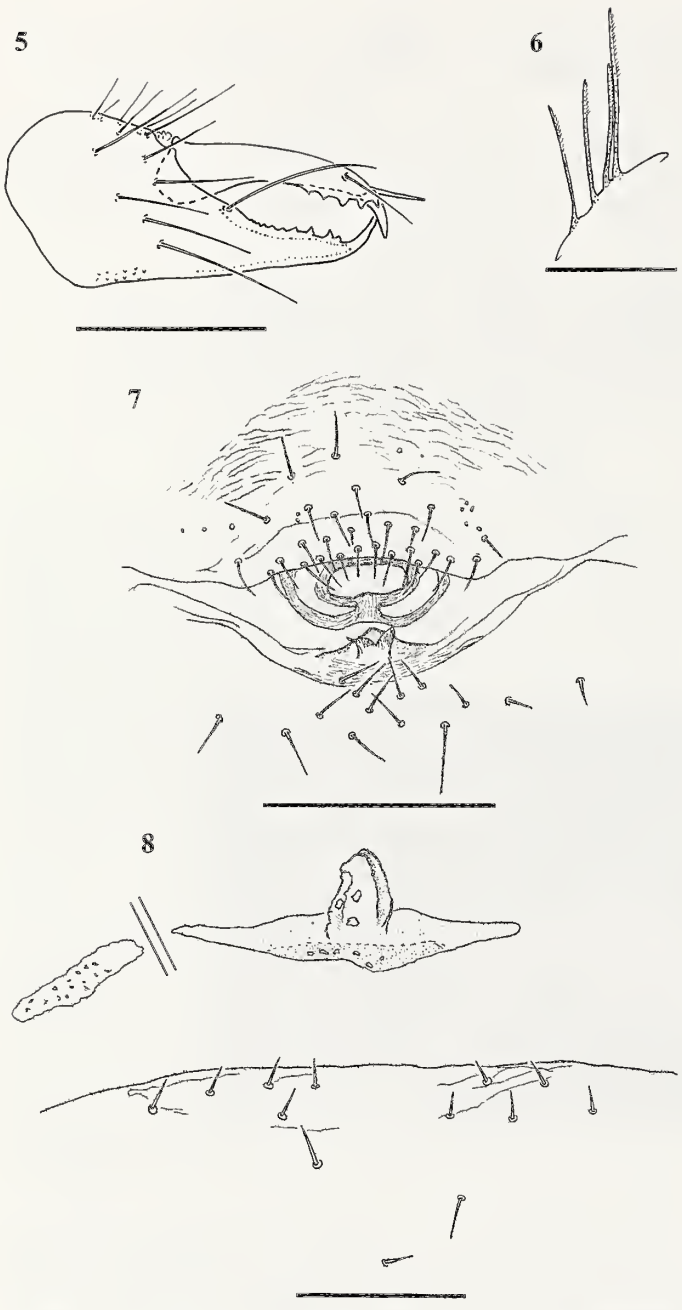
Genital operculum of females (Fig. 8): 12 marginal and discal short setae (♀: 13), median cribrate plate sclerotized, with a median finger-like prolongation, lateral cribrate plates elongate.

Chaetotaxy of female sternites: III with 14 marginal setae (♀: 16) and 3 suprastigmal setae on each side, IV: 7–8 + 2×2, V–VII: 11–12, VIII–X: 11, XI: 6, slit-like pores present. Anal cone of all specimens with 2 pairs of short setae.

Genital operculum of males: (Fig. 7) with 17–18 marginal short setae and approximately 11 longer discal setae, sternite II medially somewhat furrowed with tiny pores.

Chaetotaxy of male sternites: III: 5–7 marginal longer setae, 7 median discal setae + 6 setae on sternite (2 suprastigmal setae on each side), IV: 9–10, V: 10, VI: 9–11, VII: 11, VIII: 11–12, IX: 11, X: 10–11, XI: 6.

Pedipalps: (Figs. 9, 10, 11, 17; see Table 1 for measurements and proportions). Trochanter finely granulate, indistinct hump present; femur with a distinct tubercle on lateral side near end, with a series of tooth-like small protuberances, finely granulate, patella with a medial protuberance near end of



Figures 5–8.—*Spelaeobochica iuiu* new species: 5. Left chelicera of female holotype (scale bar 0.3 mm); 6. Rallum of female holotype (scale bar 0.1 mm); 7. Genital operculum of male paratype (scale bar 0.25 mm); 8. Genital operculum of female holotype and one median and one lateral cribrate plate (scale bar 0.1 mm).

pedicel, with a few tooth-like protuberances on medial side and mediodistally finely granulate, hand with pedicel very finely granulate in distal half, on medial side near base of fixed finger with a tooth-like protuberance. Fixed finger (holotype: distal third of the left palpal finger missing) with about 75–78 broad, acute, densely set teeth, in distal half of finger 8–9 antiaxial accessory teeth present, 19–22 paraxial accessory teeth; with total of 27–30 accessory teeth. Movable finger with 73–79 teeth, movable finger with 5–6 (right)/4–8 (left) antiaxial and 13–16 (right)/11–13 (left) paraxial accessory teeth (partly in two series), with total of 15–21 accessory teeth. Venom apparatus well developed in both fingers, venom duct long, nodus ramosus slightly distal to *ist*, respectively *st*. Trichobothrial pattern: *ib* in distal half of hand dorsum; *ist* slightly distal of *est*, *est* is proximate compared to *st* on movable finger, *isb* in basal position much nearer to *esb* than to level of *sb*, *it* indistinctly distal of *et*; *b-sb-st-t* almost equidistant.

Leg I (Fig. 12): see Table 1 for measurements and proportions.

Leg IV (Figs. 13, 19): see Table 1 for measurements and proportions. Subterminal setae finely dentate apically (Fig. 14); arolia undivided and shorter than smooth claws.

Description of tritonymph.—Paler than adult (whitish coloration) with the fingers of pedipalps and chelicerae light red. Vestitural setae smooth, delicate, and long.

Carapace: 1.5 times longer than broad; with 40 setae (6 setae on anterior margin and 6 on posterior margin).

Chelicerae: 8 acuminate dorsal and 2 lateroventral setae on hand, fixed finger with 9 rounded teeth, movable finger with 4 rounded teeth and a large, laterally displaced subterminal tooth, similar to adults; galea slender; acute; subgaleal seta reaching end of galea; serrula exterior with 25 blades, serrula interior with 18 blades; rallum of 4 apically dentate blades (with the same aspect of the rallum of adults).

Tergites undivided, chaetotaxy: 4: 4: 6: 6: 6: 6: 9: 9: 9: 6: 6. Pleural membranes smoothly, longitudinally striate; manducatory process acute, with 2 apical marginal and 1–2 discal setae, 1 short seta on palpal coxa; palpal coxa laterally scaly, sculptured, with tiny pores, 6 long setae (shorter than in adults), coxae also with tiny pores (number decreasing from I to IV), I with 4 setae, II 3, III 3, IV 4.

Sternites chaetotaxy: 4: 7: 6: 9: 10: 10: 11: 11: 11: 6. Anal cone with 2 pairs of short setae.

Pedipalps: see Table 1 for measurements and proportions. Trochanter slightly and finely granulate (granulation less than adult), indistinct hump present; femur with a distinct tubercle on lateral side near end, with some subtle tooth-like very small protuberances, patella with a medial protuberance near pedicel ending, with a few small tooth-like protuberances on medial side and mediodistally finely granulate, on medial side near base of fixed finger with a tooth-like protuberance. Trichobothria *sb* and *ist* absent; fixed finger with 47 teeth, 3 antiaxial and 10 paraxial accessory teeth, movable finger with 46 teeth, antiaxial accessory teeth lacking, 7 paraxial accessory teeth.

Leg I: see Table 1 for measurements and proportions. Basitarsus and telotarsus with a less visible suture line.

Leg IV: see Table 1 for measurements and proportions. Subterminal setae similar to adults. Basitarsus and telotarsus with a less visible suture line.

KEY TO SPECIES OF *SPELAEOBOCHICA*

1. Rallum of 4 dentate blades, eyeless, but with two tiny, lateroventral tubercles on each side of carapace. Pedipalps: femur 4.2 times longer than broad; chela with pedicel 4.1 times. Leg IV: femur+patella 3.8 times longer than deep . . . . . *S. allodentatus*

Table 1.—Measurements (mm) and proportions (length/breadth) of body parts of *Spelaeobochica iuiu* new species.

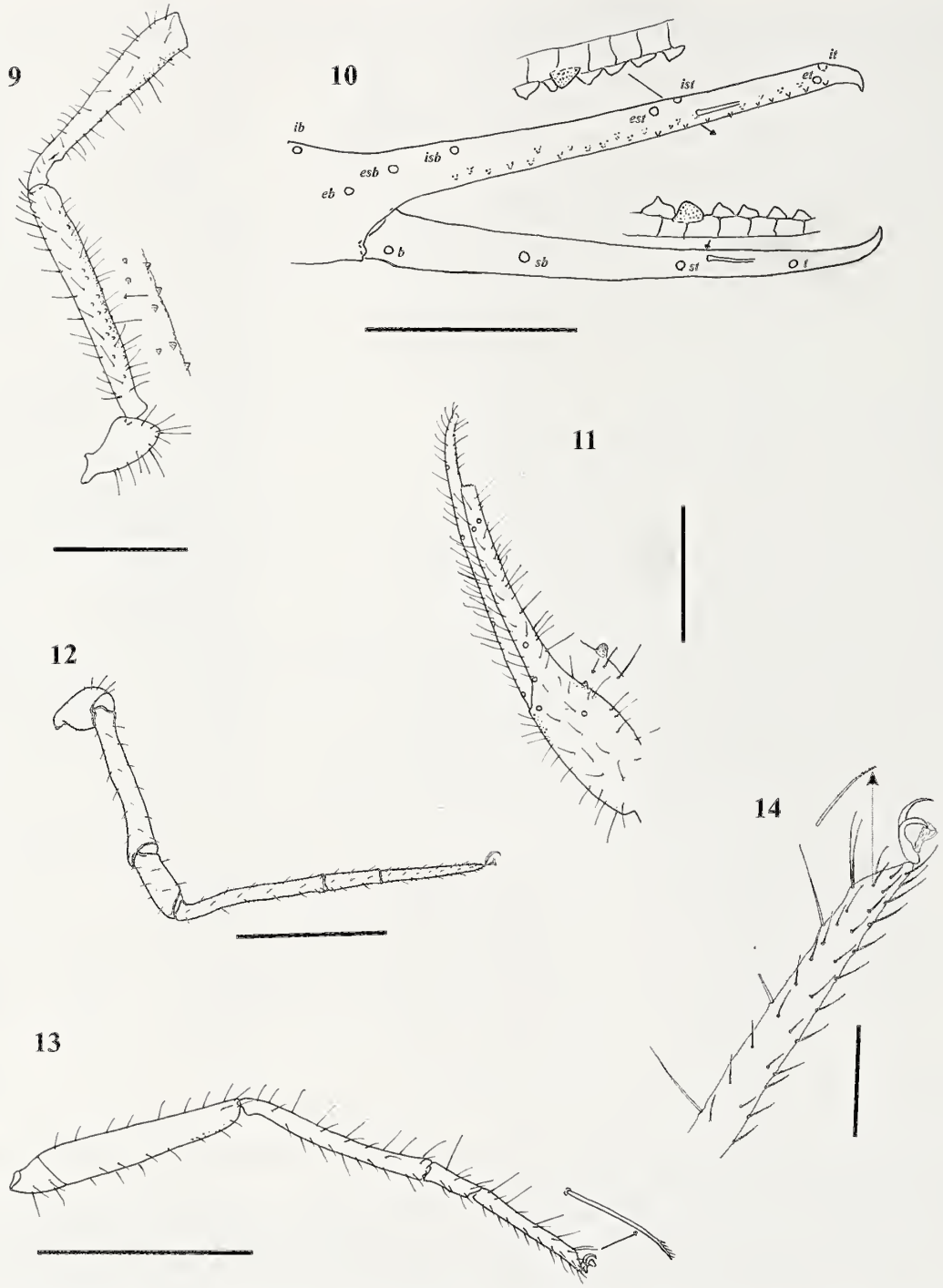
	♀ holotype	♂ paratypes	♀ paratype	tritonymph paratype
Body	5.17	4.47–4.34	5.29	3.2
Carapace	1.44/0.95	1.40/0.85–1.32/0.84	1.48/1.00	0.95/0.65
	1.5×	1.6×	1.5×	1.5×
Pedipalp				
Trochanter	0.81/0.39	0.67/0.39–0.60/0.37	0.80/0.41	0.41/0.32
	2.1×	1.7–1.6×	2.0×	1.3×
Femur	2.16/0.28	2.08/0.25–2.00/0.24	2.20/0.28	1.32/0.20
	7.8×	8.3×	7.9×	6.6×
Patella	2.00/0.34	1.92/0.29–1.80/0.30	2.00/0.36	1.16/0.24
	5.9×	6.6–6.0×	5.6×	4.8×
Hand with pedicel	1.17/0.53	1.16/0.50–1.08/0.45	1.20/0.48	0.77/0.38
	2.2×	2.3–2.4×	2.5×	2.0×
Finger length	2.13	2.08–1.96	2.28	1.36
Finger/hand with pedicel	1.8×	1.8×	1.9×	1.8×
Chela with pedicel	3.21	3.12–2.96	3.32	2.08
	6.1×	6.2–6.6×	6.9×	5.5×
Leg I				
Femur	0.99/0.18	0.95/0.21–0.90/0.16	1.04/0.18	0.58/0.11
	5.5×	4.5–5.6×	5.8×	5.3×
Femur/patella	2.3×	2.4–2.3×	2.4×	2.2×
Patella	0.43/0.16	0.40/0.19–0.39/0.15	0.44/0.15	0.26/0.12
	2.8×	2.1–2.6×	2.9×	2.2×
Tibia	0.95/0.10	0.89/0.11–0.80/0.09	0.94/0.10	0.52/0.08
	9.2×	8.0–8.9×	9.4×	6.5×
Basitarsus	0.33/0.09	0.30/0.10–0.28/0.08	0.33/0.09	0.18/0.07
	3.9×	3.0–3.5×	3.7×	2.6×
Telotarsus	0.62/0.07	0.61/0.09–0.55/0.07	0.59/0.07	0.38/0.06
	8.3×	6.8–7.9×	8.4×	6.3×
Basitarsus/telotarsus	1.9×	2.0×	1.8×	2.1×
Leg IV				
Femur+patella	1.63/0.27	1.52/0.30–1.48/0.24	1.60/0.38	1.00/0.19
	6.1×	5.1–6.2×	4.2×	5.3×
Tibia	1.35/0.14	1.28/0.14–1.16/0.17	1.32/0.10	0.81/0.09
	9.6×	9.1–6.8×	13.2×	9.0×
Basitarsus	0.37/0.13	0.35/0.13–0.35/0.10	0.36/0.09	0.22/0.08
	2.9×	2.7–3.5×	4.0×	2.8×
Telotarsus	0.87/0.09	0.84/0.11–0.80/0.09	0.89/0.08	0.54/0.08
	9.3×	7.6–8.9×	11.1×	6.8×
Basitarsus/telotarsus	2.4×	2.4–2.3×	2.5×	2.5×

Rallum of 4–6 dentate blades, eyeless, without tubercles on each side of carapace, pedipalps and legs much more slender. Leg IV femur+patella greater than 4.0 times longer than deep 2

2. Rallum of 4 dentate blades. Medial side of hand near base of fixed finger with a tooth-like protuberance. Pedipalps: femur 7.8–8.3 longer than broad; chela with pedicel 6.1–6.9 times longer than broad. Leg IV: femur+patella 4.2–6.2 times longer than deep . . . . . *S. iuiu*
Rallum of 6 dentate blades. Medial side of hand near base of fixed finger without a tooth-like protuberance. Pedipalps: femur 12.5 times longer than broad; chela with pedicel 8.6 times longer than broad. Leg IV: femur+patella 9.5 times longer than deep . . . . *S. muchmorei*

Habitat.—The Lapa do Baixão cave comprises the only known locality of this species. The cave was not totally explored since part of its inner chambers becomes flooded during rainy periods. However, the known conduits extend over 500 meters. The specimens were restricted to the inner portions of the cave in a conduit that becomes partially submerged during the rainy season; they were found walking freely on the floor and walls, both covered by a layer of fine silty sediment. During the first incursion to the cave (20 July 2007), we found only one tritonymph; three years later (8 June 2010), we found a total of 10 specimens (all adults). Potential prey include mites, springtails, juvenile crickets (*Endecous* sp., Phalangopsidae) and diplopods (Polydesmida, Oniscodesmidae). The only known entrance to the cave is located at the bottom of a subsidence sinkhole, which receives epigean water, especially during strong rain. The external area is severely impacted, mainly by human activities such as agriculture and extensive breeding of cattle and goats. Fortunately, the cave has not been visited by anyone except the research team.

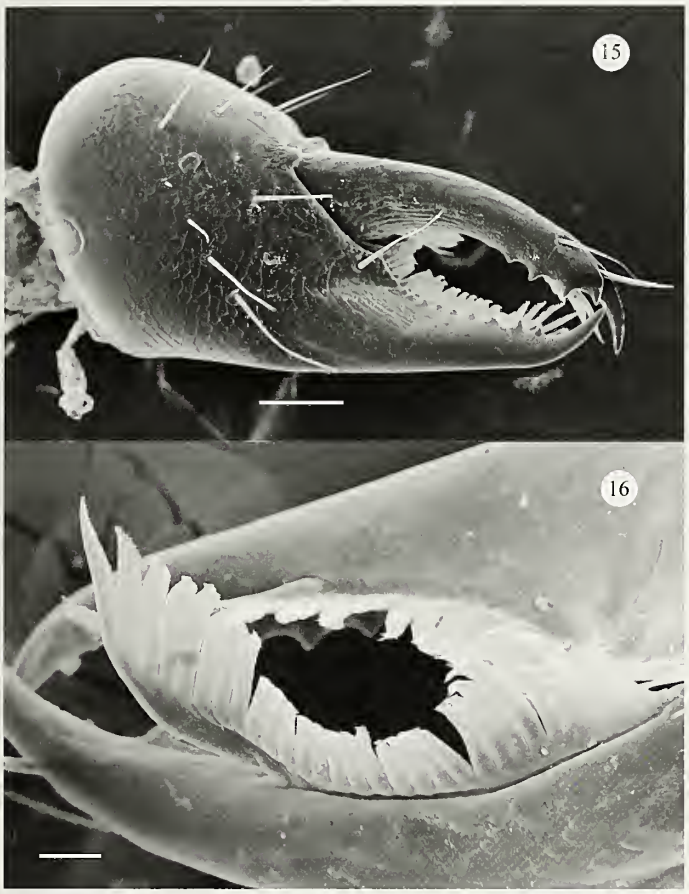
Discussion.—The new species displays the main characters of the genus *Spelaeobochica*: 4–6 setae in rallum, high number of setae on the cheliceral hand, venom apparatus well developed in both chelal fingers, distal position of trichobothrium *ib*, presence of accessory teeth on the chelal fingers,



Figures 9–14.—*Spelaeobochica iuiu* new species: 9. Left pedipalp of female holotype, small protuberances magnified (scale bar 1.00 mm); 10. Right chela of holotype lateral view, marginal tooth rows omitted, replaced by detail drawings (scale bar 1.0 mm); 11. Left chela of female holotype in dorsal view, tooth-like protuberance magnified (scale bar 1.0 mm); 12. Leg I of female holotype (scale bar 1.00 mm); 13. Leg IV of female holotype (scale bar 1.0 mm); 14. Detail of telotarsus of leg IV showing subterminal seta (scale bar 0.25 mm).

and the presence of a distinct tubercle on the palpal femur. Two species have been previously described in this genus, *S. muchmorei* from caves of São Paulo (Mahnert 2001) and *S. allodentatus* from Bahia (Gruta do Impossível, Palmeiras) (Andrade & Mahnert 2003) (Fig. 20). *Spelaeobochica iuiu* can be easily distinguished from those species by the presence of tooth-like protuberances on the pedipalps and by its measurements. It is distinctly smaller than *S. muchmorei* (e.g., femur length 2.76–3.32 mm vs 2.0–2.16 mm) with stouter pedipalps (e.g., femur 12.5–13.3 times as long as broad vs 7.8–8.3 times), but it is distinctly larger than *S. allodentatus* (e.g., femur length 0.95–0.97 mm vs 2.0–2.16 mm) with more slender pedipalps (e.g., femur 4.2 times as long as broad vs. 7.8–8.3 times). Furthermore, the pedipalps are smooth in *S. allodentatus*, but granulate in *S. iuiu*.

Until 2008, all natural cavities in the Brazilian territory were protected by law. Unfortunately, the national legislation was



Figures 15–16.—*Spelaeobochica iuiu* new species, scanning electron microscope images: 15. Left chelicera of female paratype (scale bar 0.1 mm); 16. Detail of serrula exterior on left chelicera of female paratype (scale bar 0.03 mm).

altered after this date. Nowadays, many different anthropogenic activities (especially mining) occur in karst areas, and caves all over the country are being damaged. Therefore, government officials created categories (based on biological and geological parameters) to define the "status" of each cave intending to determine which could be sacrificed and which should be preserved. To assure the complete conservation of a cave in Brazil, it is necessary from a biological point of view to prove the occurrence of at least one endemic troglobitic species. Approximately 30 caves have been surveyed by biologists in the state of Bahia (Pinto-da-Rocha 1995). Our research team went to eight cavities near Lapa do Baixão cave, and found no specimens of *S. iuiu*. This strongly suggests that the species is endemic to Lapa do Baixão cave. Furthermore, the description of *S. iuiu*, besides contributing to the knowledge of pseudoscorpion diversity in the Neotropics, ensures the protection of the cave.

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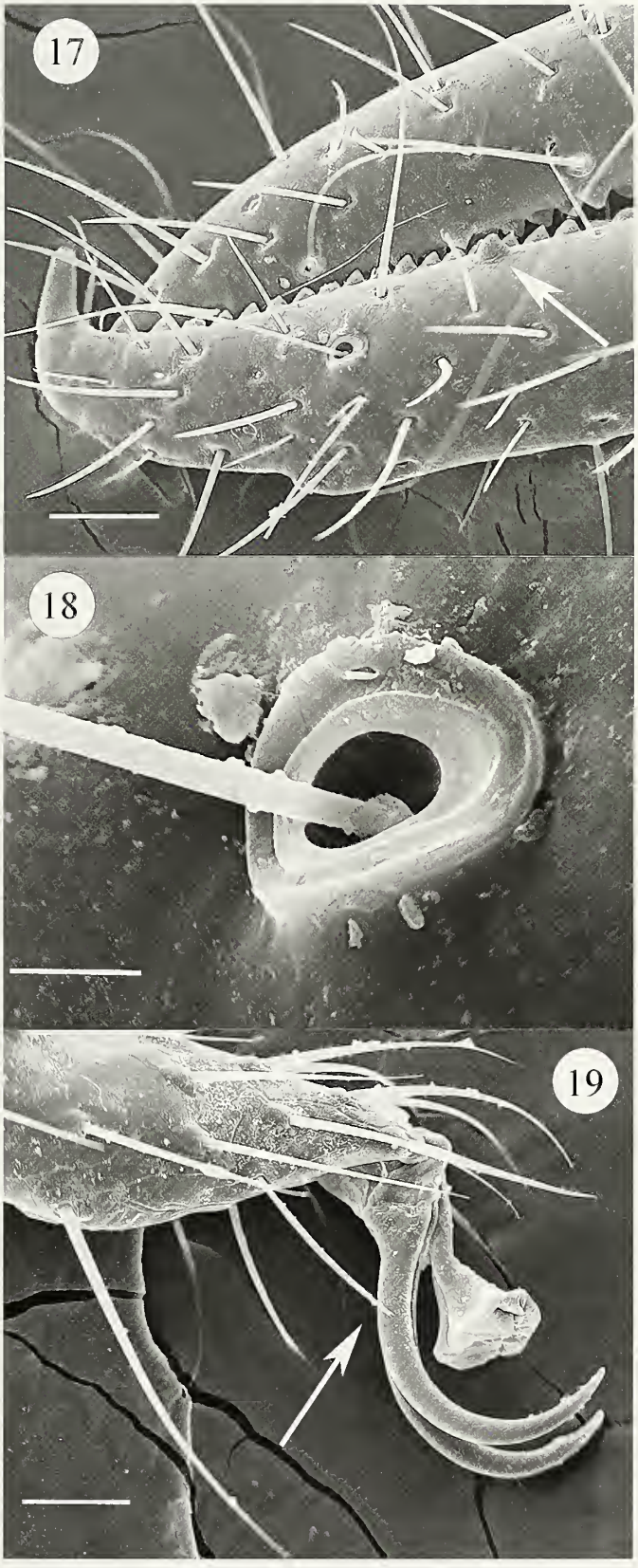

Figures 17–19.—*Spelaeobochica iuiu* new species, scanning electron microscope images: 17. Left chela of female paratype, arrow indicating accessory tooth (scale bar 0.04 mm); 18. Detail of trichobothria *it* of female paratype (scale bar 0.02 mm); 19. Detail of leg IV of female paratype, arrow pointing at subterminal setae (scale bar 0.04 mm).

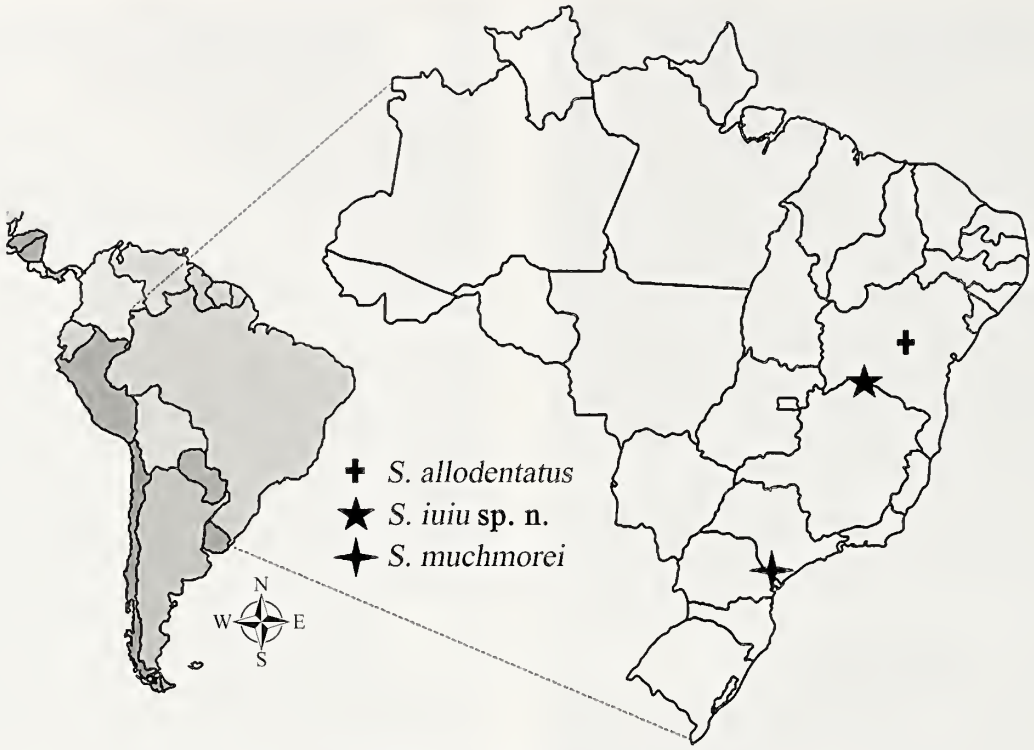

Figure 20.—Distribution map of *Spelaeobochica* in Brazil.

suggestions, and especially to all the staff of the Laboratory of Subterranean Ecology (Zoology Sector-UFLA). We also thank the Conselho Nacional do Desenvolvimento Científico e Tecnológico (CNPq 301061/2011–4). Sincere thanks are addressed to the three reviewers for their critical and constructive comments.

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Multivariate methods support the distinction of a new highland *Vaejovis* (Scorpiones: Vaejovidae) from the Sierra de los Ajos, Mexico

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Abstract: Multivariate analyses of morphological characters provide strong evidence that a highland *Vaejovis* from the Sierra de los Ajos, a Madrean 'sky island' in northern Sonora, Mexico, represents a distinct new species of the *V. vorhiesi* group. This new species is described and compared to other geographically adjacent species of the *V. vorhiesi* group, named *V. bandido*, and brief notes on ecology are provided. Results from this study provide evidence that multivariate analysis of morphological characters is a powerful tool to delimit small and otherwise cryptic scorpion species.

Keywords: Madrean pine-oak, scorpion, taxonomy, *Vaejovis mexicanus* group, *Vaejovis vorhiesi* group

Recent studies continue to reveal distinct new species of *Vaejovis* in the highlands of western North America (Graham 2007; Ayrey 2009; Graham & Bryson 2010; Ayrey & Soleglad 2011; Sissom 2011). The *V. vorhiesi* group, a related assemblage of these highland scorpions, is distributed throughout pine-oak-juniper woodlands from Utah south across Arizona and western New Mexico into northern Mexico (Sissom 2000, 2011). Until recently (Santibáñez-López & Francke 2010), the *V. vorhiesi* group was considered part of the *V. mexicanus* group, which includes other highland forms distributed in Mexico and western Texas (McWest 2009). As currently understood, the *V. vorhiesi* group consists of the following species: *V. vorhiesi* Stahnke 1940, *V. jonesi* Stahnke 1940, *V. lapidicola* Stahnke 1940, *V. paysonensis* Soleglad 1973, *V. cashi* Graham 2007, *V. feti* Graham 2007, *V. deboerae* Ayrey 2009, *V. crumpi* Ayrey & Soleglad 2011, *V. bigelowi* Sissom 2011 and *V. electrum* Hughes 2011. Although geographically proximate to these species and previously assigned to the *V. vorhiesi* group (Graham & Bryson 2010), preliminary genetic data suggest that *V. vaquero* Gertsch & Soleglad 1972 and *V. montanus* Graham & Bryson 2010 are not members of this group (L. Prendini pers. comm.).

The distribution of the *V. vorhiesi* group is often cited as including northern Mexico (e.g., Sissom 2000; Santibanez-Lopez & Francke 2010; Sissom 2011), but other than *V. montanus* and *V. vaquero*, no other montane *Vaejovis* have been documented from this region. The 'sky islands' of northern Mexico, together with those of adjoining southern Arizona and New Mexico, form an archipelago of mesic mountain habitat flanked by arid desert and grassland plains. Because of their isolation, the remote Sierra de los Ajos and other Madrean sky islands in Mexico, such as the Sierra El Tigre, may harbor distinct new highland species of scorpions.

We surveyed the Sierra de los Ajos in October of 2010. One day and two nights of searching the upper elevations of the Sierra de los Ajos revealed a large population of a small montane species of the *V. vorhiesi* group. We collected nine individuals, and measured morphological characters for comparison to *V. vorhiesi* group forms from six nearby mountain ranges. Measurements were analyzed using multivariate statistical analyses. Our results indicated that the scorpions from the Sierra de los Ajos represent a distinct new species, which we describe here. This study, as well as similar research on related species (Hughes 2011), suggests that multivariate analysis of morphological characters appears to be an effective strategy when delimiting small and otherwise cryptic montane vaejovid species.

METHODS

Sampling.—We scored 23 morphological characters from 54 female *Vaejovis* from seven different mountain ranges, including the Animas Mountains, New Mexico; Santa Catalina Mountains, Arizona (*V. deboerae*); Chiricahua Mountains, Arizona (*V. cashi*); Huachuca Mountains, Arizona (*V. vorhiesi*); Peloncillo Mountains, New Mexico; Sierra Elenita, Sonora; and Sierra de los Ajos, Sonora. Because sexual dimorphism in scorpions is strong, sexes should be analyzed separately. We therefore excluded male specimens due to inadequate sample sizes from several ranges. Female *Vaejovis* appear to be more morphologically conserved than males, which vary in size and chelal morphology, and based on studies of other scorpion taxa (e.g., van der Meijden 2010), this variation likely represents an adaptive difference between sexes. By measuring females only, we assumed that differences detected between groups most likely represented selectively neutral morphological divergence, which is arguably a better indicator of phylogeny, and thus species limits, than traits strongly biased by selection. All characters were measured with an ocular micrometer.

Morphology.—Measurements are as described by Stahnke (1970), pedipalp finger dentition follows Soleglad & Sissom (2001), trichobothrial patterns are as in Vachon (1974) and Soleglad & Fet (2003) and hemispermatophore terminology is from Soleglad & Fet (2008). Terminology follows Stahnke (1970) and Stockmann & Ythier (2010). For measurements, the words "length," "width," and "depth" are abbreviated as L, W, and D. We measured total lengths from the anterior margin of the carapace to the aculeus tip, with the telson fully extended.

Statistical analyses.—Multivariate statistical assessment largely followed Devitt et al. (2008), with a few notable modifications. We began by performing a principal component

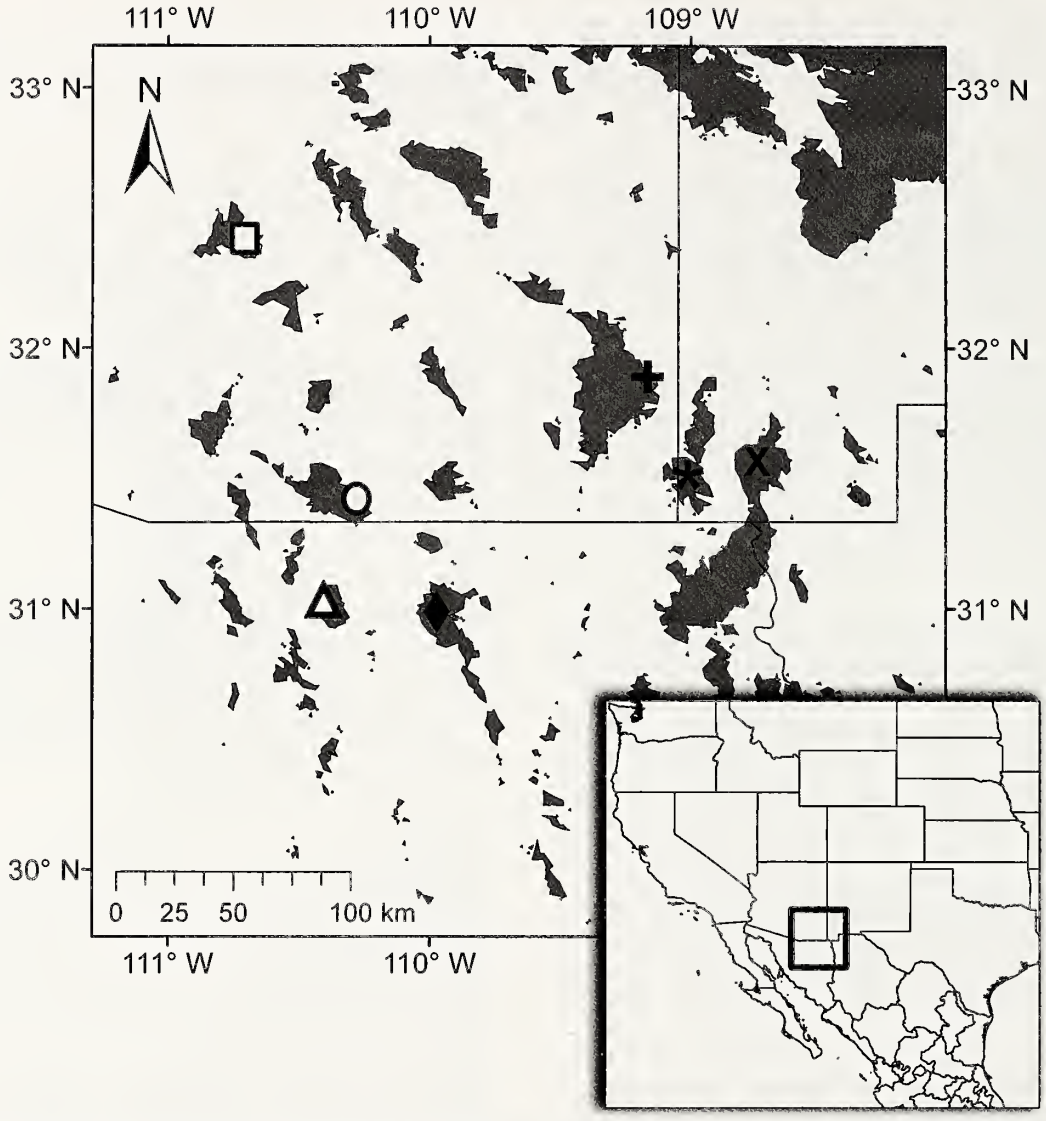


Figure 1.—Distribution of scorpions in the *Vaejovis vorhiesi* group from the southwestern 'sky island' region that were included in this study. Gray areas indicate elevations of 1,650 m and above. Symbols represent populations from the Santa Catalina Mountains (*V. deboerae*, square), Huachuca Mountains (*V. vorhiesi*, circle), Sierra Elenita (*V.* cf. *vorhiesi* triangle), Sierra de los Ajos (*V. bandido* new species, diamond), Chiricahua Mountains (*V. cashi*, cross), Peloncillo Mountains (*V.* cf. *cashi*, asterisk), and the Animas Mountains (*V.* cf. *cashi*, X). Symbols correspond to those used in Fig. 2.

analysis (PCA) to explore patterns of variation in the data and to delimit any natural groupings in the dataset, using mountain range as the grouping variable. Scatterplots were constructed for each pair of principal components and used to check for outliers. We then performed standard univariate descriptive statistics for each of the non-overlapping groupings identified by the PCA (mean ± 1 standard deviation, range, sample size), and used ANOVA to compare means. For post-hoc comparisons of means, we used Scheffé's F, which is robust to violations of the assumption of homogeneity of variances (Scheffé 1953; Devitt et al. 2008).

To determine which variables contribute the most to the disparity between groups, we then performed a discriminant function analysis (DFA), again using the natural non-overlapping groups specified by the PCA as the grouping variable. While PCA searches for the directions of highest variation without consideration of group membership, DFA examines variation among groups compared to the variability within them, thereby detecting those variables responsible for group differentiation. Because DFA can sometimes be sensitive to deviations from normality, we explored the effect of standardization and log transformation of non-normal data. Neither transformation noticeably altered the results, so we performed a final DFA on the raw data. Significance of canonical functions was assessed using a classification matrix with jackknife validation. We performed all statistical analyses with SYSTAT® v. 8.0 Statistics (SPSS Inc. 1998).

Abbreviations used in multivariate analyses are as follows: CARA_L = carapace length, METI_L = length of metasoma segment I, METI_W = width of metasoma segment I, METII_L = length of metasoma segment II, METII_W = width of metasoma segment II, METIII_L = length of metasoma segment III, METIII_W = width of metasoma segment III, METIV_L = length of metasoma segment IV,

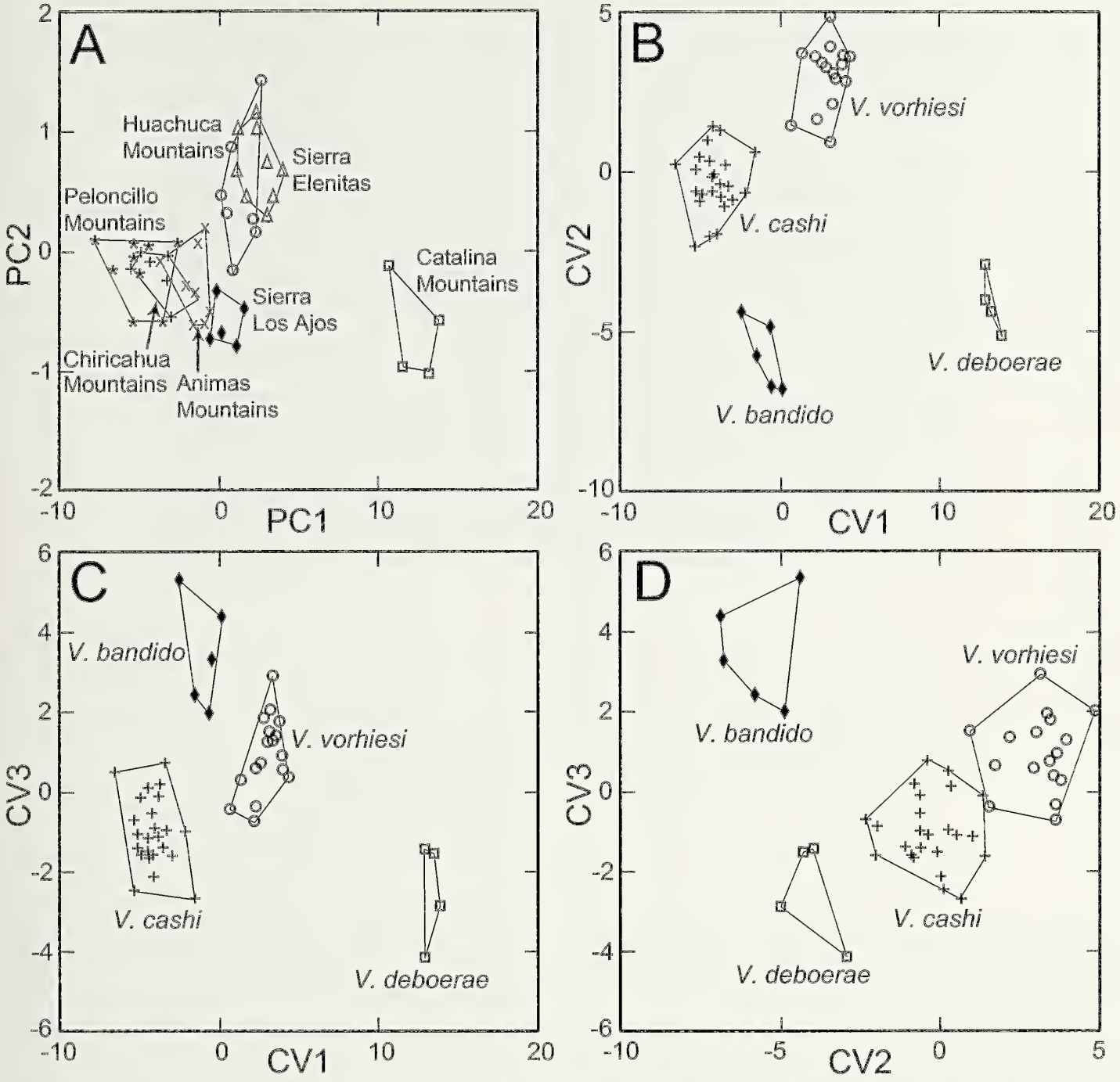


Figure 2.—Scatterplots of principal components (A) and canonical analysis of discriminance (B–D) for female 'sky island' scorpions. Individuals were grouped by mountain range in the PCA, and natural clusters were assigned to one of four species used as the grouping variable in the DFA.

METIV_W = width of metasoma segment IV, METV_L = length of metasoma segment V, METI_W = width of metasoma segment V, VES_L = length of telson vesicle, VES_W = width of telson vesicle, VES_D = depth of telson vesicle, FEM_L = femur length, FEM_W = femur width, PAT_L = patella length, PAT_W = patella width, PALM_L = length of chelal palm, PALM_W = width of chelal palm, PALM_D = depth of chelal palm, MF_L = length of pedipalp movable finger and FF_L = length of pedipalp fixed finger.

Acronyms of depositories.—AMNH, American Museum of Natural History; CAS, California Academy of Sciences; CNAN, Colección Nacional de Arácnidos, Instituto de Biologia, Universidad Nacional Autónoma de México, México, D.F.; UANL, Universidad Autónoma de Nuevo León, San Nicolas de los Garza, Nuevo León, Mexico; MRG, personal collection of Matthew R. Graham, Las Vegas, Nevada, USA; RFA, personal collection of Richard F. Ayrey, Flagstaff, Arizona, USA.

Table 1.—Morphological variation in females of four species in the *Vaejovis vorhiesi* group: *V. bandido* new species, *V. cashi*, *V. deboerae*, and *V. vorhiesi*. For each character, the mean ± standard deviation and range are provided in the first row. Values in the second row of each character indicate species with significant ($P < 0.05$) pairwise difference in means (species coded 1–4), as indicated from ANOVAs using Scheffé's F post-hoc procedure. Character abbreviations are defined in the Methods. Sample sizes are listed in the first row.

	V. bandido (1)	*V. cashi (2)*	*V. deboerae (3)*	*V. vorhiesi (4)*
N	5	24	4	17
CARA_L	3.23±0.10 (3.12–3.38) 2, 3	2.80±0.22 (2.33–3.14) 1, 3, 4	4.30±0.17 (4.12–4.48) 1, 2, 4	3.38±0.15 (3.07–3.62) 2, 3
METI_L	1.38±0.06 (1.31–1.45) 2, 3, 4	1.23±0.10 (1.00–1.38) 1, 3, 4	2.05±0.05 (2.00–2.12) 1, 2, 4	1.54±0.07 (1.41–1.67) 1, 2, 3
METI_W	1.814±0.04 (1.77–1.86) 2, 3	1.57±0.11 (1.33–1.76) 1, 3, 4	2.43±0.08 (2.33–2.50) 1, 2, 4	1.88±0.08 (1.79–2.00) 2, 3
METIII_L	1.68±0.08 (1.57–1.79) 2, 3	1.46±0.11 (1.24–1.67) 1, 3, 4	2.46±0.09 (2.41–2.60) 1, 2, 4	1.85±0.09 (1.69–2.00) 2, 3
METIII_W	1.68±0.06 (1.62–1.74) 2, 3	1.46±0.10 (1.21–1.62) 1, 3, 4	2.22±0.06 (2.14–2.26) 1, 2, 4	1.69±0.06 (1.60–1.81) 2, 3
METV_L	3.32±0.13 (3.17–3.50) 2, 3	2.79±0.21 (2.31–3.10) 1, 3, 4	4.59±0.17 (4.38–4.76) 1, 2, 4	3.47±0.15 (3.26–3.69) 2, 3
METV_W	1.58±0.04 (1.55–1.62) 2, 3	1.37±0.09 (1.14–1.52) 1, 3, 4	2.06±0.10 (1.93–2.17) 1, 2, 4	1.57±0.05 (1.50–1.62) 2, 3
VES_L	1.82±0.04 (1.76–1.86) 2, 3	1.58±0.10 (1.31–1.71) 1, 3, 4	2.55±0.09 (2.50–2.69) 1, 2, 4	1.95±0.13 (1.69–2.17) 2, 3
VES_W	1.11±0.05 (1.01–1.17) 2, 3	0.98±0.07 (0.83–1.07) 1, 3, 4	1.45±0.10 (1.36–1.55) 1, 2, 4	1.12±0.07 (0.98–1.24) 2, 3
FEM_L	2.54±0.08 (2.45–2.64) 3	2.25±0.22 (1.81–2.60) 3, 4	3.71±0.16 (3.52–3.86) 1, 2, 4	2.84±0.12 (2.67–3.14) 2, 3
FEM_W	0.86±0.03 (0.83–0.91) 3	0.78±0.07 (0.64–0.88) 3, 4	1.18±0.05 (1.12–1.24) 1, 2, 4	0.91±0.04 (0.86–0.98) 2, 3
PAT_L	2.82±0.14 (2.69–3.05) 2, 3	2.44±0.22 (2.02–2.81) 1, 3, 4	3.96±0.18 (3.79–4.14) 1, 2, 4	3.05±0.13 (2.83–3.33) 2, 3
PAT_W	0.95±0.01 (0.93–0.95) 2, 3	0.84±0.06 (0.74–0.98) 1, 3, 4	1.33±0.05 (1.26–1.38) 1, 2, 4	0.99±0.04 (0.93–1.07) 2, 3
PALM_L	2.31±0.07 (2.21–2.41) 2, 3	2.01±0.18 (1.67–2.29) 1, 3, 4	3.32±0.14 (3.19–3.50) 1, 2, 4	2.48±0.08 (2.33–2.69) 2, 3
PALM_D	1.12±0.04 (1.07–1.17) 2, 3	0.97±0.09 (0.81–1.10) 1, 3, 4	1.61±0.08 (1.52–1.71) 1, 2, 4	1.11±0.07 (0.98–1.24) 2, 3
MF_L	2.71±0.11 (2.60–2.88) 3	2.37±0.26 (1.95–2.79) 3, 4	4.05±0.16 (3.91–4.29) 1, 2, 4	3.09±0.13 (2.86–3.38) 2, 3
FF_L	2.16±0.09 (2.05–2.29) 3, 4	1.9±0.22 (1.52–2.24) 3, 4	3.33±0.13 (3.26–3.52) 1, 2, 4	2.55±0.12 (2.33–2.83) 1, 2, 3

Material examined (other than types).—*Vaejovis bandido:* MEXICO: *Sonora*, Sierra de los Ajos, 30.98017°N, 109.96685°W, 1840 m, 12–13 October 2010, R.W. Bryson, Jr., 3 ♂ (1 CNAN, 2 UANL), 3 ♀ (1 CNAN, 2 UANL).

Vaejovis cashi Graham 2007: USA: *Arizona*, Cochise Co., Herb Martyr Canyon, Chiricahua Mountains, 31.8901°N, 109.1686°W, 1530 m, 15 March 2008, R.W. Bryson, Jr., 8 ♀ (MRG).

Vaejovis cf. *cashi:* USA: *New Mexico*: Hidalgo Co., Geronimo Pass, Peloncillo Mountains, 31.51725°N, 109.016917°W, 1718 m, 16 September 2010, R.W. Bryson, Jr., 6 ♂, 11 ♀ (MRG). Animas Mountains, 31.56433°N, 108.74416°W, 1835 m, 17 September 2010, R.W. Bryson, Jr., 1 ♂, 15 ♀ (MRG).

Vaejovis deboerae Ayrey 2009: USA: *Arizona*, Pima Co., Mount Lemon, Santa Catalina Mountains, 32.2313°N, 110.4145°W, 2142 m, 25 August 2008, R.F. Ayrey, 1 ♂, 6 ♀ (CAS).

Vaejovis vorhiesi Stahnke 1940: USA: *Arizona*, Cochise Co., Miller Canyon, Huachuca Mountains, 31.2497°N, 110.1657°W, 1757 m, 26 April 2009. R.F. Ayrey, 6 topotypes ♀ (RFA).

Vaejovis cf. *vorhiesi:* USA: *Arizona*, Santa Cruz Co., Cave Creek Canyon, Santa Rita Mountains, 31.7130°N, 110.8241°W, 1890 m, 20 March 2008, R.W. Bryson, Jr., 1 ♂, 3 ♀ (MRG). MEXICO: *Sonora*, Sierra Elenita, 31.02329°N, 110.37881°W, 1815 m, 14 October 2010, R.W. Bryson, Jr., 1 ♂, 9 ♀ (UANL).

RESULTS

PCA of female morphological characters revealed four non-overlapping groups that sorted geographically (Fig. 2). Female *V. cashi* from the Chiricahua Mountains grouped with specimens from the nearby Animas and Peloncillo Mountains, so we treated specimens from these three mountain ranges as *V. cashi*. Female scorpions from the Sierra Elenita clustered with those from the nearby Huachuca Mountains, so we treated specimens from those two ranges as *V. vorhiesi*. To the north, *V. deboerae* from the Santa Catalina Mountains formed the most distinct cluster in principal component space, and were treated as an individual species. Lastly, although close to *V. cashi* in component space, specimens from the Sierra de los Ajos also formed a non-overlapping group. Importantly, the means of these two

Figure 3.—*Vaejovis bandido* new species in life.

groups clearly separated in component space, so we treated specimens from the Sierra de los Ajos as a distinct new species, *V. bandido*, described below.

The majority of the variance (92.6%) was explained by PC1, with all factor loadings greater than 94%. Since the data were raw measurements and not ratios, which are more typical of taxonomic work on vaejovid scorpions, PC1 corresponded to variation associated with differences in size. PC2 then can be interpreted as variation associated with shape, although this component only explains 1.4% of the total variance. Therefore, PCA results suggested that *V. deboerae* differed from the other three species in overall size. This is clearly evident in raw values as adult female *V. deboerae* all ranged between 33.17 and 33.9 mm in total length, while total lengths of the other three species ranged only from 18.43 to 28.05 mm. The remaining three species were much more similar in PCA space, but varied from each other on both size (PC1) and shape (PC2) axes. PCA results suggested that female *V. bandido* were morphologically most similar to *V. cashi*, but differed in terms of overall size.

Canonical analysis of discriminance revealed highly significant differences among the four species (Wilks' $\lambda = 0.001$, $P < 0.0001$; Fig. 2B–D). Again, *V. deboerae* was the most distinct species in multivariate space, but all four species clearly segregated, with no overlapping points. *Vaejovis deboerae* was most distinct on canonical variate I (CV1), which was most strongly correlated with lengths of metasomal segments I to III and with telson width. The remaining three species differentiated most on CV2, which was most strongly explained by variation in the length of metasomal segment I, the length and width of metasomal segment II, and the width of the telson vesicle. Interestingly, although *V. bandido* overlapped considerably with *V. cashi* on CV1, it was clearly distinct on CV2 and CV3 (Fig. 2B–D). Length of metasomal segment V had the greatest factor loading on CV3, followed by femur length, metasomal segment I length, and metasomal segment II width. The classification function correctly classified 100% of the samples, and the jackknife validation correctly classified between 75% and 96% of the specimens (Table 3). Univariate statistics and ANOVA results based on these four species groupings are provided in Table 1.

TAXONOMY

Family Vaejovidae Thorell 1876
Genus *Vaejovis* Koch 1836

Vaejovis Koch 1836:51.

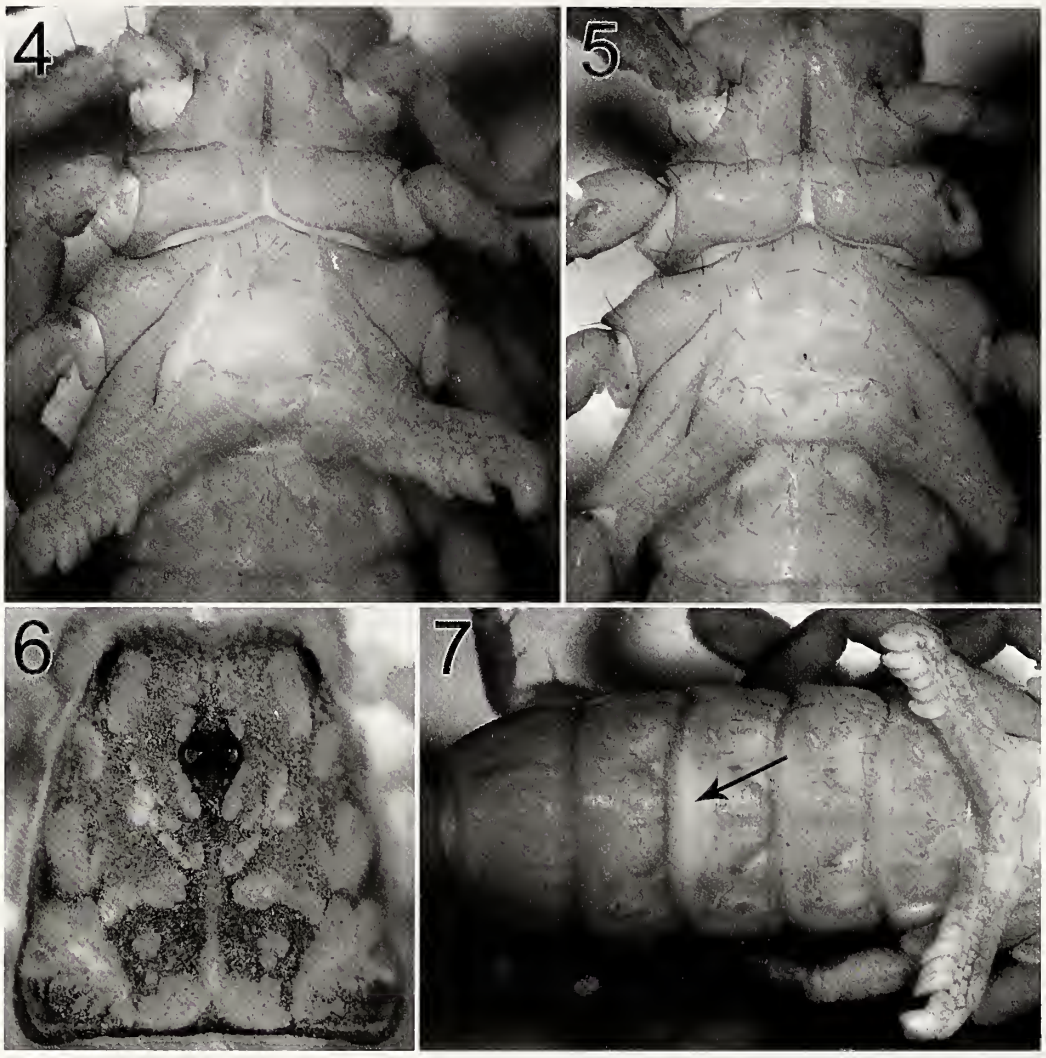
Type species.—*Vaejovis mexicanus* Koch 1836, by monotypy.

Vaejovis bandido new species
(Figures 3–18, Tables 1–3)

Type material.—MEXICO: *Sonora*, male holotype, Sierra de los Ajos, 30.98017°N, 109.96685°W, 1840 m, 12–13 October 2010, R.W. Bryson, Jr. (AMNH). Paratypes: same collection data as holotype, 2 ♀ (1 AMNH, 1 UANL).

Etymology.—The specific epithet refers to the historical use of the Sierra de los Ajos and surrounding mountains as hideouts for bandits ("*bandidos*" in Spanish) and outlaws during the early 1900s (Knight 1990).

Diagnosis.—Small in overall size with males smaller than females. Base color brown with darker mottling on the carapace, tergites, pedipalps, metasoma and legs. Median carinae of tergites I–VII obsolete. Moderate subaculear spine. Chelal tricobothria *ib* and *it* located at the base of the fixed finger; anterior margin of carapace slightly emarginated. Fixed finger ID denticles 5 and movable finger ID denticles 6, which distinguish *V. bandido* from *V. bigelowi, V. crumpi, V.*



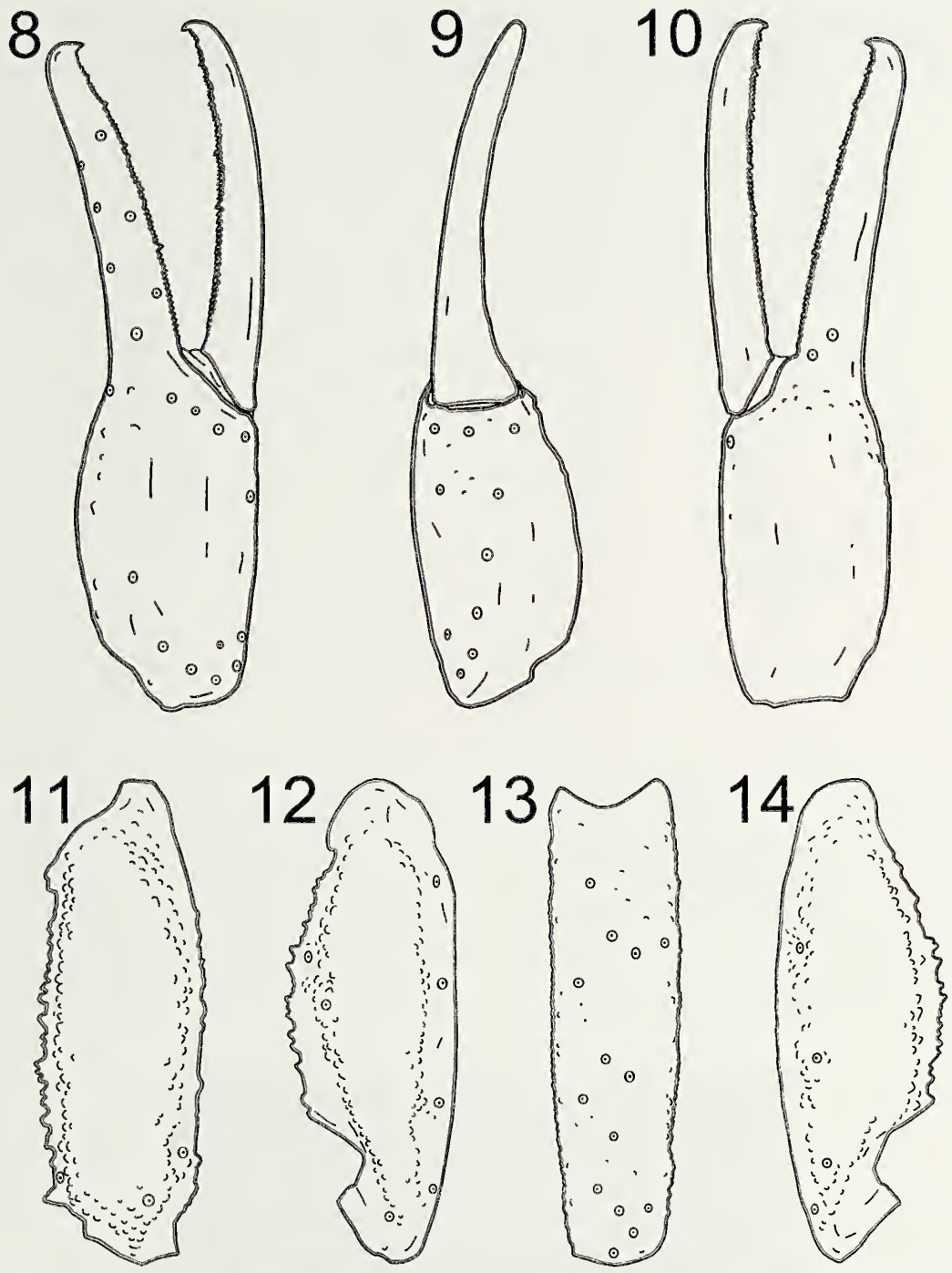
Figures 4–7.—*Vaejovis bandido* new species. 4. Pectines and sternum, male holotype. 5. Pectines and sternum, female paratype. 6. Carapace, male holotype. 7. White patch (arrow), an area of reduced pigmentation, on sternite V, male holotype.

jonesi, V. lapidicola, V. paysonensis, V. montanus, V. vaquero, and *V. mexicanus* group scorpions, which have 7 movable finger ID denticles. Single pair of distal spinules on ventral surface of leg tarsi, not 3 or more as in *V. vaquero* and all *V. mexicanus* group species (Santibanez-Lopez & Francke, 2010), and not 2–3 as in *V. montanus*. Differs from *V. electrum* by smaller body size and a palm width of 0.95–1.07 mm in females, instead of 1.05–1.28 in females. Trichobothrium *Db* ventral to *D1* carinae on chelal palm. Pectine count 13–14 in males and 11–12 in females.

Adult females can be distinguished from adult female *V. vorhiesi, V. cashi, V. feti*, and *V. deboerae* by the following ratios that overlap by less than 10%, as outlined in Hughes (2011): pedipalp fixed finger length/palm width, 19.6–2.19 in *V. bandido* and 2.35–3.03 in *V. vorhiesi* (0% overlap); pedipalp chela length/width, 4.09–4.45 in *V. bandido* and 4.67–5.78 in *V. vorhiesi* (0% overlap); metasomal segment V length/segment I length, 2.34–2.45 in *V. bandido* and 2.18–2.36 in *V. cashi* (7.4% overlap); chelal palm length/width, 2.10–2.16 in *V. bandido* and 1.95 in *V. feti* holotype; 2.13–2.31 in *V. bandido* and 1.68 in *V. feti* holotype; metasomal segment V length/width, carapace length/palm width 3.00–3.17 in *V. bandido* and 2.72–3.03 in *V. deboerae* (6.7% overlap). *Vaejovis bandido* can also easily be distinguished from *V. deboerae* by a conspicuous difference in overall size, the latter species being the larger. In the female specimens examined, carapace lengths, a commonly used proxy for overall size, ranged from 3.12 to 3.38 mm in *V. bandido* and from 4.12 to 4.18 mm in *V. deboerae*. In addition, female type specimen total lengths were much smaller for *V. bandido*: 27.75 mm compared to 33.14 and 32.21 mm in *V. deboerae* (Ayrey 2009). These differences, as well as other size characters in which *V. bandido* differs from *V. cashi, V. deboerae,* and *V. vorhiesi*, are summarized in Table 1.

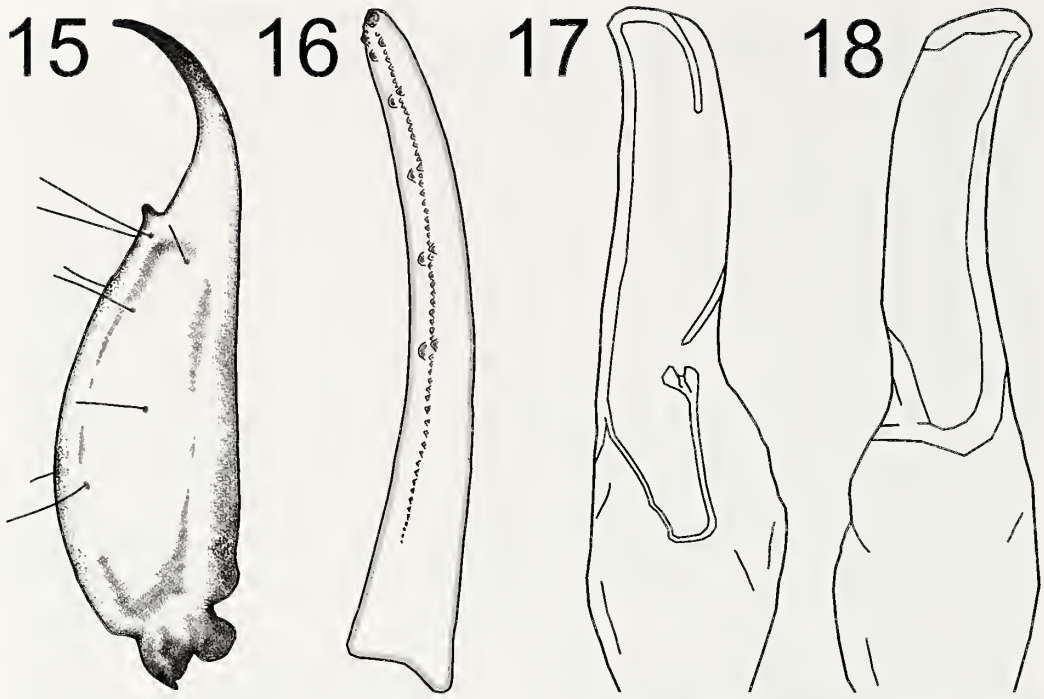
Description of holotype.—*Color:* Base color brown with darker mottling on the carapace, tergites, pedipalps, metasoma and legs (Figs. 3–7). White patch on the posterior 1/3 of sternite V (Fig. 7), similar to *V. deboerae, V. montanus*, and *V. bigelowi*.

Morphology: Carapace: anterior margin slightly emarginate, with three lateral eyes on each side; moderately convex dorsolaterally; finely granular with scattered small granules; median furrow moderate and traversing length of carapace, excluding the median eyes; ratio of median eyes location (distance from anterior edge)/carapace L = 0.33; carapace L/W at



Figures 8–14.—Trichbothrial patterns of *Vaejovis bandido* new species, based on male holotype. 8. Right pedipalp chela, external. 9. Right pedipalp chela, ventral. 10. Right pedipalp chela, internal. 11. Right pedipalp femur, dorsal. 12. Right pedipalp patella, dorsal. 13. Right pedipalp patella, external. 14. Right pedipalp patella, ventral.

median eyes = 1.46. Tergites: coarsely granular, median carinae obsolete; strong granular submedian and lateral carinae on posterior 4/5s of VII; pretergites very finely granular. Genital operculum: sclerites separated on posterior 1/5. Pectines: tooth count 13/13; sensorial areas present on all teeth (sensorial areas present on all teeth in females, but slightly reduced on the most basal tooth): middle lamellae 7/7. Sternites: III–VI smooth to very finely granular and without carinae; VII with granular lateral carinae on posterior 1/2. Spiracles: ovoid with median side rotated 35 degrees away from posterior sternite margin. Metasoma: ratio of segment I L/W 0.65; segment II L/W 0.94; segment III L/W 1.02; segment IV L/W 1.45; segment V L/W 2.18. Segments I–IV: dorsolateral carinae strong, serrate with distal denticle enlarged and spinoid; lateral supramedian carinae strong with serrated granules and enlarged spinoid distal denticle; lateral inframedian carinae moderate, granular on segment I, distal 1/2 of II and III, and obsolete on IV; ventrolateral carinae strong, granular; ventral submedian carinae weak, granular on I and II, on III weak basally and moderate distally, moderate



Figures 15–18.—*Vaejovis bandido* new species, male holotype. 15. Telson, lateral view. 16. Movable finger of right pedipalp. 17. Right hemispermatophore, ectal view. 18. Right hemispermatophore, ental view.

on IV; dorsal and lateral intercarinal spaces very finely granular; ventrolateral setation 2/2:1/2:2/2:3/3; ventral submedian setation 3/3:3/3:3/3:3/3. Segment V: dorsolateral carinae moderate, distally crenulate, basally granular; lateromedian carinae moderate and granular on basal 3/4, obsolete on distal 1/4; ventrolateral carinae moderate and serrate; ventromedian carinae moderate, granular; intercarinal spaces finely granular; dorsolateral setation 2/2; lateromedian setation 3/3; ventrolateral setation 4/4; ventromedian setation 3/3. Telson: smooth, with prominent subaculear spine; LAS denticles (Fet et al. 2006) lacking. Chelicerae: dorsal edge of movable cheliceral finger with two subdistal (sd) denticles; ventral edge smooth with well developed serrula comprised of approximately 20 tines on distal half. Pedipalps: trichobothrial pattern type C (Figs. 8–14); ratio of chela L/W = 4.33; femur L/W = 2.88; patella L/W = 2.93; fixed finger L/carapace L = 0.64. Chela: carinae weak with some weak to moderate granules; median (MD) denticles of fixed finger aligned and divided into six subrows by five outer (OD) denticles; flanked by five inner (ID) denticles; movable finger with six subrows,

Figure 19.—Madrean pine-oak forest riparian habitat at the type locality of *Vaejovis bandido* new species, Sierra de los Ajos, Sonora, Mexico.

Table 2.—Factor loadings for the first and second principal components and canonical discriminant functions for the first, second, and third canonical variate scores. See Methods for character abbreviations.

	PC1	PC2	CV1	CV2	CV3
CARA_L	0.989	0.040	0.035	0.548	0.287
METI_L	0.989	0.103	2.039	2.896	−2.177
METI_W	0.986	−0.008	−0.371	0.284	0.029
METII_L	0.989	0.110	−2.294	−1.594	0.754
METII_W	0.984	−0.129	0.661	−1.665	−2.166
METIII_L	0.988	0.117	1.360	0.239	−1.483
METIII_W	0.984	−0.102	−0.736	0.724	−0.020
METIV_L	0.988	0.115	−0.260	0.002	−0.074
METIV_W	0.976	−0.154	0.879	−1.305	0.992
METV_L	0.994	0.044	0.094	−1.263	4.065
METV_W	0.979	−0.144	−0.630	−0.160	0.388
VES_L	0.971	0.108	0.742	0.116	−0.246
VES_W	0.968	−0.088	−1.451	−1.656	1.497
VES_D	0.982	0.019	0.740	1.128	−1.710
FEM_L	0.987	0.116	−0.046	−1.240	−2.286
FEM_W	0.978	−0.054	−1.091	1.050	−0.324
PAT_L	0.990	0.086	0.310	1.046	−0.025
PAT_W	0.980	−0.102	1.627	1.108	−0.060
PALM_L	0.992	0.022	0.045	−0.593	1.712
PALM_W	0.947	−0.235	−0.885	0.988	−1.528
PALM_D	0.966	−0.202	0.267	−1.774	−0.114
MF_L	0.982	0.143	−1.375	−3.630	1.145
FF_L	0.977	0.178	1.057	4.685	1.034
Eigenvalue	22.141	0.327	27.602	8.563	2.725
Total Variance (%)	96.264	1.421	–	–	–
Total Dispersion (%)	–	–	0.71	0.93	1

five OD denticles and six ID denticles; movable finger shorter than both the carapace and metasomal segment V. Femur: dorsoexternal, dorsointernal, and ventrointernal carinae crenulate, ventroexternal weak, granular. Patella: internal carinae oblique and granular; all other carinae moderate, crenulate. Legs: ventral surface of tarsus with single median row of spinules terminating distally with one spinule pair. Hemispermatophore (Figs. 17 & 18): Lamelliform type with well-developed distal lamina with a distinct distal crest about 1/3 the length of the lamina. Slight basal constriction located just proximal of lamina midpoint where it terminates in well-developed, bifurcated, lamellar hook. A sclerotized spermatocleutrum (= mating plug) was not found in either hemispermatophore, although it is possible that they could have been inadvertently removed during the dissection process. We did not find a mating plug in two additional males that were dissected.

Mensuration (mm).—Male holotype: total L = 24.35; carapace L = 2.93; mesosoma L = 6.86; metasoma L = 10.64 (excluding telson). Metasoma: segment I L/W = 1.43/1.76; segment II L/W = 1.67/1.69; segment III L/W = 1.76/1.62; segment IV L/W = 2.38/1.57; segment V L/W = 3.40/1.55. Telson: L = 2.95; vesicle L/W/D = 1.90/1.02/0.83; aculeus L = 1.05. Pedipalps: total L = 7.17; femur L/W = 2.43/0.83; patella L/W = 2.67/0.90; chela L = 4.05; palm L/W/D = 2.07/0.98/1.10; movable finger L = 2.43; fixed finger L = 1.98. Female paratype 1: total L = 26.10; carapace L = 3.21; mesosoma L = 9.52; metasoma L = 10.19 (excluding telson); Metasoma: segment I L/W = 1.36/1.79; segment II L/W = 1.60/1.71; segment III L/W = 1.69/1.62; segment IV L/W = 2.26/1.62; segment V L/W = 3.29/1.55. Telson: L = 2.93; vesicle L/W/D = 1.81/1.07/0.83; aculeus L = 1.12. Pedipalps: total L = 9.62; femur L/W = 2.48/0.86; patella L/W = 2.76/0.95; chela L = 4.38; palm L/W/D = 2.29/1.07/1.12; movable finger L = 2.60; fixed finger L = 2.10. Female paratype 2: total L = 27.75; carapace L = 3.17; mesosoma L = 9.00; metasoma L = 10.07 (without telson); Metasoma: segment I L/W = 1.36/1.79; segment II L/W = 1.55/1.69; segment III L/W = 1.64/1.67; segment IV L/W = 2.26/1.57; segment V L/W = 3.26/1.55. Telson: L = 2.90; vesicle L/W/D = 1.81/1.07/0.79; aculeus L = 1.12. Pedipalps: total L = 9.76; femur L/W = 2.55/0.86; patella L/W = 2.76/0.93; chela L = 4.45; palm

Table 3.—Classification matrix from the discriminant function analysis of female scorpions in the *Vaejovis vorhiesi* group. Jack-knife validation values that differed from those of the classification function are in parentheses.

Species	*V. bandido*	*V. cashi*	*V. deboerae*	*V. vorhiesi*	%correct
V. bandido	5 (4)	0 (1)	0	0	100 (80)
V. cashi	0	24 (23)	0	0 (1)	100 (96)
V. deboerae	0	0	4 (3)	0 (1)	100 (75)
V. vorhiesi	0	0 (1)	0	17 (16)	100 (94)
Total	5 (4)	24 (25)	4 (3)	17 (18)	100 (92)

L/W/D = 2.31/1.00/1.07; movable finger L = 2.74; fixed finger L = 2.19.

Variability.—Sexual dimorphism in *V. bandido* was strong. Of the adult specimens examined, the average female total length was 26.04 mm (n = 5), while the average male total length was 21.86 mm (n = 4). Characters that differed in size most between the sexes were as follows: carapace length 2.48–2.93 mm in males and 3.17–3.38 mm in females (0% overlap); mesosoma length 5.9–6.86 mm in males and 7.29–9.52 mm in females (0% overlap); patella length 2.29–2.52 mm in males and 2.76–3.05 mm in females (0% overlap); chela length 3.52–4.05 mm in males and 4.38–4.69 mm in females (0% overlap). The sexes also differed in the shape of the metasoma, which can be most easily seen in ratios of metasomal segment III length/width; 2.10–2.16 in females and 2.18–2.23 in males (0% overlap). Although there was some size variation within the sexes, intraspecific variation in shape (measured as ratios) appeared to be exceptionally low.

Distribution.—Known only from the type locality in the Sierra de los Ajos of northern Sonora, Mexico (Fig. 1).

Ecology.—Specimens were collected 12–13 October 2010. During diurnal searches, *V. bandido* were frequently found beneath small rocks along a riparian corridor at 1840 m (Fig. 19). This riparian area was covered in thickets of pine and oak trees, and much of the ground was covered with bunchgrass and oak leaf litter. Only two specimens were found under the same rock, and the vast majority of the scorpions encountered under rocks were female. At night, *V. bandido* were found active on the surface in the same habitat. During this time, scorpions, including numerous males, were found in large numbers along rock and dirt banks above the road.

To roughly estimate the local abundance of *V. bandido*, we conducted a one-hour survey on 13 October 2011 along a 0.8 km stretch of dirt road that paralleled the riparian area. From 1930 h to 2030 h, three people methodically searched the ground adjacent to the road with portable UV lights while a fourth person recorded observations. Care was taken to avoid counting the same scorpion more than once. During this time, 149 *V. bandido* were observed.

Additional scorpions observed in the area were *Centruroides sculpturatus* Ewing 1928, *Vaejovis spinigerus* Wood 1863, and *Diplocentrus* cf. *spitzeri* Stahnke 1970. With the exception of *C. sculpturatus*, most of these were found on the drier rocky slopes above the riparian area. Here, *D.* cf. *spitzeri* seemed most abundant, although no specific counts were made. Eight *C. sculpturatus* were observed during the one-hour nocturnal transect, and several were found under rocks within the riparian habitat.

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A new species of *Heterolacurbs* (Opiliones: Biantidae: Stenostygninae) from Puerto Rico

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Abstract. A new species of Biantidae belonging to the genus *Heterolacurbs* Roewer 1912 is herein described. *Heterolacurbs perezassoi* new species from Puerto Rico, Greater Antilles, is the second species included in the genus, and it is clearly recognized by the pair of large spiniform apophyses on area IV that does not restrain area III of the dorsal scutum, the smooth legs, femora II–IV without dorsodistal spine, its tarsal formula, sternites with small tubercles and penis that exhibits a distinctive morphology.

Keywords: Laniatores, Samooidea, taxonomy, West Indies

The genus *Heterolacurbs* Roewer 1912 currently comprises a single species, *H. ovalis* Roewer 1912. *Heterolacurbs* can be recognized by the presence on area IV of a pair of large spiniform apophyses and free tergite III with a medial spiniform apophysis. It also may be distinguished from all other stenostygnid genera by the penial morphology, a capsula interna composed by an apically pointed stylus, lateroapically flattened and wide, flanked by two basally fused conductors, apically with laminar lobes.

Roewer (1912) originally placed *Heterolacurbs ovalis* as part of the Biantinae in the then numerous family Phalangodidae. Mello-Leitão (1938) elevated it to family status. Lawrence (1959) erected the subfamily Lacurbsinae for the western tropical African biantids, including *H. ovalis* by implication. Martens (1978) excluded *Heterolacurbs* from Biantidae, but did not reassign it to another family. Staręga (1992) restored it to Biantidae, but did not specify which subfamily. Pérez-González & Alegre (2009) established that *H. ovalis* Roewer 1912 mislabelled as from Togo, Africa is a senior synonym of *Martibianta virginsulana* Šilhavý 1973 from United States Virgin Islands (West Indies) and removed the genus *Heterolacurbs* from Lacurbsinae to the Neotropical subfamily Stenostygninae. Armas (2010) recorded the family Biantidae from Puerto Rico for the first time and cited it as a new species here described of *Heterolacurbs*, being the second known species for the genus. This new species is described herein and provides data on its intraspecific variability, habitat, natural history and distribution.

The specimens studied are lodged in the arachnological collection of the Institute of Ecology and Systematics (CZACC), Havana, Cuba. All measurements are given in millimeters and were made with a Carl Zeiss microscope equipped with an ocular micrometer. Abbreviations are as follows: (PL) prosoma length, (PW) prosoma width, (DSL) dorsal scutum length, (DSW) dorsal scutum width, (Fe) femur, (Mt) metatarsus, (Pa) patella, (Ta) tarsus, (Ti) tibia, (Tr) trochanter. Pedipalpal tibia and tarsus setal coding follows previous authors (Pinto-da-Rocha 1997; Acosta *et al.* 2007); *i.e.*, "i" indicates small setae (half the size of the longest setae), "I" indicates long setae and listed from basal to distal. The penial morphology nomenclature follows Kury & Pérez-González (2007). The method of male genitalia preparation and illustration follows Acosta *et al.* (2007). The penis was expanded by first placing it in lactic acid at room temperature, then heating (not boiling) for about two minutes, cooling for two minutes away from heat and rapidly transferring it to distilled water at room temperature. Line drawings were made with the software packages CorelDRAW 13 and Adobe Photoshop CS3 using photographs as templates. The map was produced with the computer GmapCatcher program using satellite images.

TAXONOMY

Family Biantidae Thorell 1889
Subfamily Stenostygninae Roewer 1913
Genus *Heterolacurbs* Roewer 1912

Type species.—*Heterolacurbs ovalis* Roewer 1912, by monotypy.

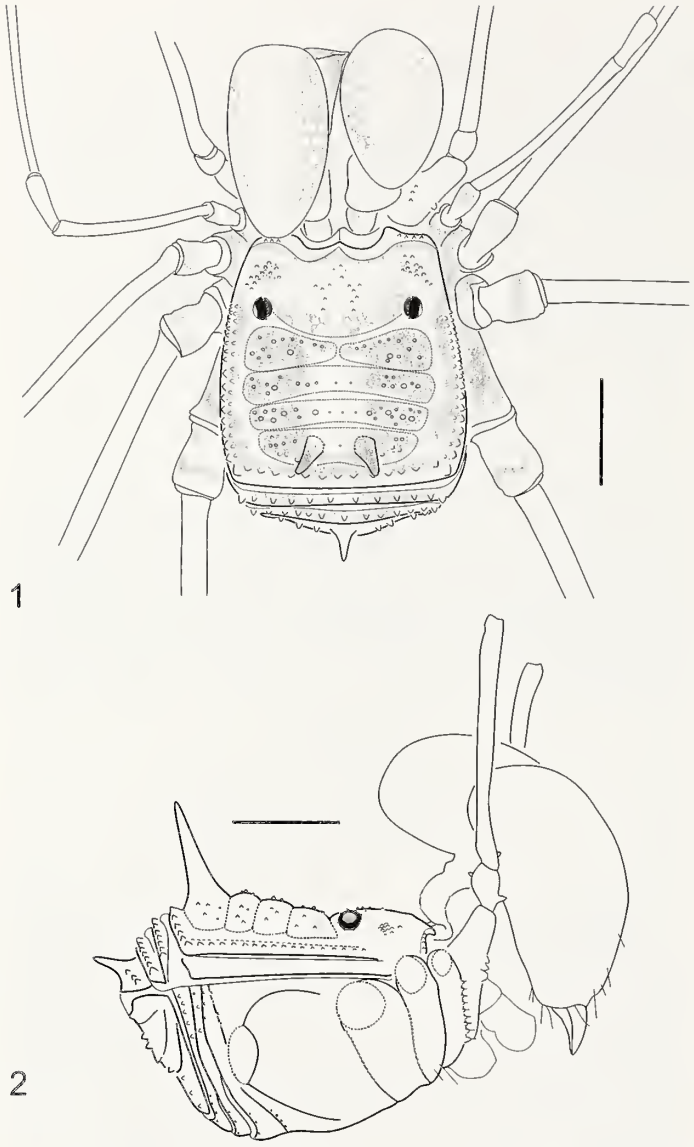
Emended diagnosis.—Dorsal scutum almost rectangular. Eye mounds with scarce small granules, near sulcus I. Area I with granules or small lateral tubercles, divided into left and right halves by a brief and narrow median groove. Area IV with a pair of large spiniform apophyses and free tergite III, with a medial spiniform apophysis. Femora II–IV with or without dorsodistal spine. Tarsal counts of leg I five or seven, leg II 9–14, leg III and IV 8–9. Penial morphology with a capsula interna composed by an apically pointed stylus, lateroapically flattened and wide, flanked by two basally fused conductors, apically with laminar lobes.

Remarks.—The penial morphology of the genus *Manahunca* points to a very close relationship with *Heterolacurbs*. Both of them present a markedly swollen pars distalis, a finger–like ventroapical process and a lateroapically flattened and wide pointed stylus. However, the apical ends of the conductors are very different between the two genera; in *Manahunca* species they are apically acute. The armature of the dorsal scutum and free tergites from both genera are also very different: the *Manahunca* species only present small tubercles.

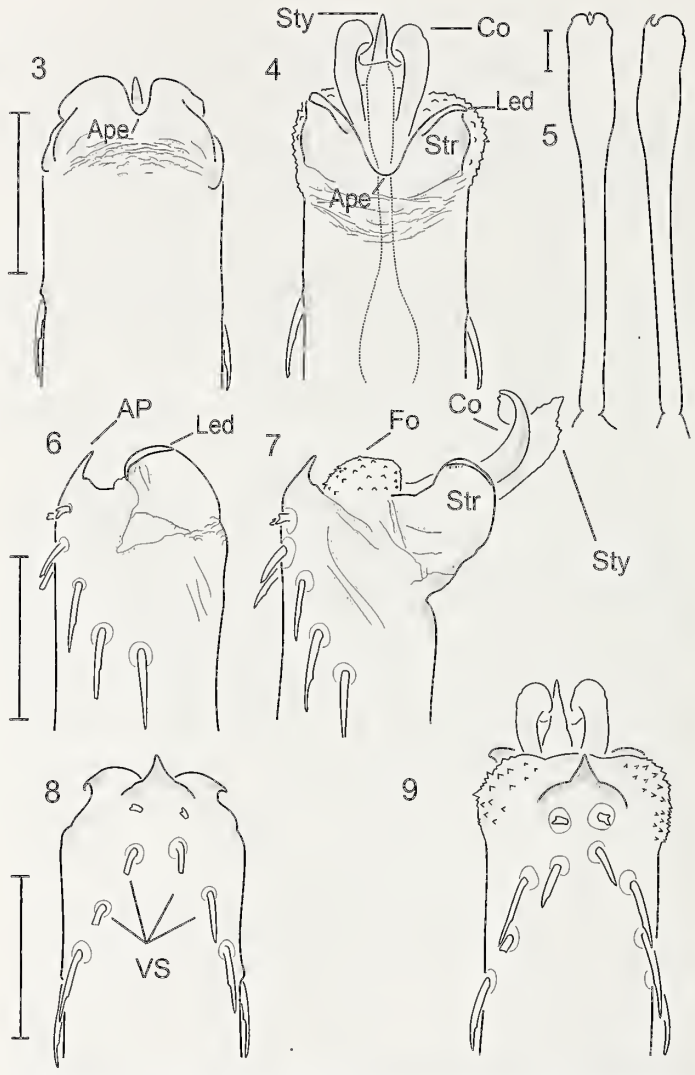
Heterolacurbs perezassoi new species
Figs. 1–10

Biantidae: Armas 2010:59, fig. 3E.
Heterolacurbs new species: Armas 2010:62.

Type material.—PUERTO RICO: holotype male, *Sierra de Guardarraya*, Barrio Los Pollos, farm at the end of road 7757, 18°00′00.8″N, 65°58′55.8″W, 170 m, 28 July 2010, L.F. Armas & A. Pérez Asso, under damp wood in yard of house (CZACC



Figures 1–2.—*Heterolacurbs perezassoi* new species, male holotype: 1. Habitus, dorsal view; 2. Habitus, lateral view. Scale = 1 mm.



Figures 3–9.—*Heterolacurbs perezassoi* new species, male holotype, distal part of penis: 3. Dorsal view; 4. Dorsal view; 5. Dorsal and lateral views, total penis; 6. Lateral view; 7. Lateral view, expanded; 8. Ventral view; 9. Ventral view, expanded. Abbreviations: Str, stragulum; Ape, aperture; Led, ledge; Co, conductors; Sty, stylus; Fo, follis; AP, apical process; VS, ventral setae. Scale = 0.1 mm.

3.3179). Paratypes: 1 male (CZACC 3.3180), 1 female (CZACC 3.3181), 1 male (CZACC 3.3182) and 1 female (CZACC 3.3183), same data as holotype.

Etymology.—The specific name honors Antonio Pérez Asso, a specialist who has worked actively with diplopods of the Antilles and has collaborated in the collection of the new species here described.

Diagnosis.—*Heterolacurbs perezassoi* new species is very close to *H. ovalis*, but is clearly distinguished from it by a pair of large spiniform apophyses on area IV, which possess a wide base, but do not restrain area III. Legs unarmed, with only scarce short sensilla, femora II–IV without dorsodistal spine, sternites with very small tubercles only, and higher tarsal formula. It also differs from *H. ovalis* in having a penis with a slightly more bulky gland, a lower ventral finger-like process, the two pairs of ventroapical setae more widely separated from each other, the most apical pair of setae reduced in size; and the stragulum with wider dorsal aperture and a thin apical ledge.

Description.—*Male holotype:* dorsal measurements: PL 0.96, PW 2.04, DSL 2.32, DSW 2.24. For measurements of appendages, see Table 1.

Dorsum (Figs. 1–2)*:* Dorsal scutum almost rectangular. Anterior margin with shallow cheliceral sockets and 3–4 small tooth-like tubercles on each side. Prosoma finely granulated in the medial region and more heavily in the anterior region of each eye mound. Eye mounds with scarce small granules, near sulcus I. Lateral margins with two rows of tubercles, the lateral row more prominent, the tubercles increasing in size toward the posterior margin. Area I with small granules and divided into left and right halves by a brief and narrow median groove. Area II finely granulated. Area III granulated with 3–4 lateral small hair tubercles. Area IV with a pair of large spiniform apophyses, each one possessing a wide base that covers the entire length of the area, but without compressing area III. Posterior margin with a row of small hair tubercles, those on each corner larger. Free tergites I–II with a row of

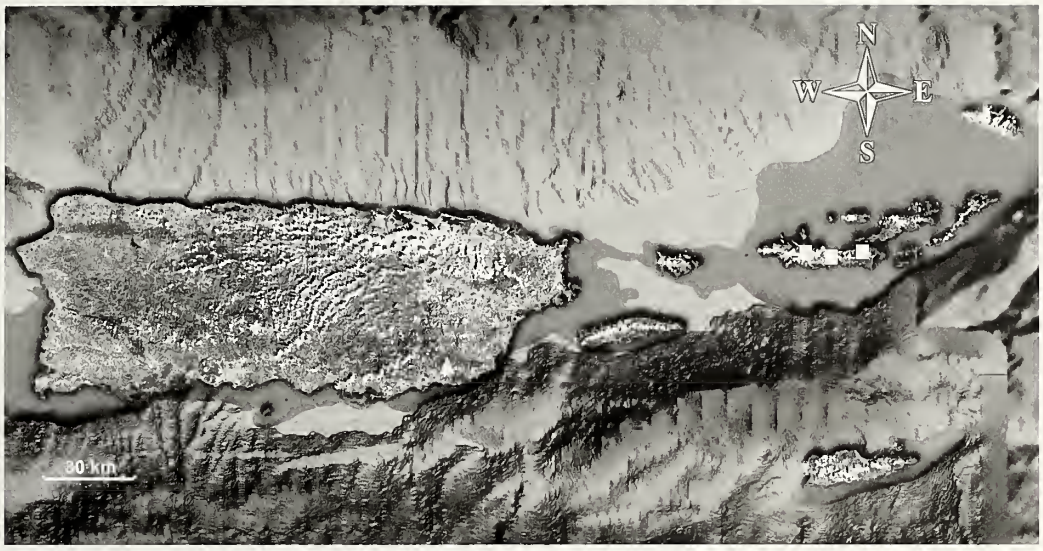


Figure 10.—Distribution of the genus *Heterolacurbs*: *H. perezassoi* new species (▲); *H. ovalis* Roewer, 1912 (■).

small hair tubercles, those on each corner being more prominent, free tergite III with a medial strong spiniform apophysis. Anal operculum with small hair tubercles.

Venter: Coxae I, III and IV with an anterior row of hair tubercles, which are larger on the first coxa. Coxa II with only one small anterolateral tubercle. Free sternites with a row of very small hair tubercles, those toward each corner being a little more evident.

Chelicerae: Basiehelicerite slightly granulated with bulla, hand greatly swollen (hypertelic) with scattered hairs concentrated mainly at the distal portion, chelicerae fingers with teeth, fixed finger with 16 small teeth and movable finger with one basal blunt tooth and 11–12 small distal teeth.

Pedipalps: Coxae dorsally with a proximal ectal tooth-like tubercle and three mesal small tubercles. Coxa ventrally with an ectal edge on whieh are 5–6 tubercles, the two most remarkable being the proximal with a tooth-like shape and the distal with one long hair. Trochanter with a small dorsal hair granule, ventrally with two hair tubercles and one ectoproximal small hair tubercle. Femur with scarce short hairs, dorsal unarmed and ventroproximal small hair tubercle. Patella enlarged in the 1/3 distal portion, dorsally with scarce small granules and a mesal ventrodistal setiferous tubercle. Tibia and tarsus with dorsal hair granules and ventrally armed with setiferous tubercles: tibia ectal: IIIIi ($1 = 3 = 4 > 2 > 5$); mesal: III ($1 = 2 = 3$), tarsus ectal: IiIii ($1 > 3 > 2 = 4 = 5$); mesal IiIii ($1 > 3 > 2 > 4 = 5$).

Legs: Smooth with scarce short hairs. Trochanter I ventrally with 2 small hair tubereles. Femora smooth. Metatarsi III–IV with two ventrodistal lateral and retrolateral spiniform projections, metatarsus III enlarged with spindle form on distal region. Tarsal claws smooth, double and lying perpendicular to the axis of the legs. Presence of dense scopula in distitarsus III and IV. Tarsal formula: 7(3): 14 (4): 9: 9.

Penis (Figs. 3–9)*:* Gland exhibiting a very wide stragulum, which articulates dorso-distally on the truncus like a jackknife and dorsally possesses a wide aperture and a distal thin ledge. When the penis is expanded, the ventral extension of the stragulum shows a spiny follis. Ventrally the truncus has a finger–like apical process and longitudinally five pairs of setae; the most apical pair is apically bifurcate and very short, the nearest pair is a little longer, and the rest of setae are longest and acuminate. The capsula interna is composed by a pointed *stylus*, dorsoventrally serrated and lateroapically flattened and wide, flanked by two basally fused conductors, with apical laminar lobes.

Coloration (in ethanol): Dorsum reddish brown with darker brown and yellowish tones. Carapace with the medial region yellowish and toward the sulcus I and lateral sides with dark brown reticules. Anterior margin with a dark brown line all over the border. Lateral and posterior margins dark brown. Areas I–IV dark brown with a lighter medial region beyond sulcus II to area IV. Surrounding zone of the mesotergal areas reddish brown. Pair of apophyses on area IV dark brown, medial apophysis on free tergite III lighter. Coxae brown with darker region at their distal portions. Trochanters yellowish with a dark brown line at the distal portion. Femora-tarsi I–III yellowish with dark brown stripes on the distal portion and lighter spots on the stripes. Femur, patella and tibia IV reddish, with the same pattern of stripes and lighter spots. Pedipalps yellowish with dorsal dark brown reticules on tibia and tarsus. Chelicerae yellowish with lateral and medial dark brown reticules; distal part of the hand and fingers reddish.

Female: similar to the male. Anterior margin with 6 tooth-like tubercles toward the each side. Pedipalp coxae dorsally with a mesal small tooth-like tubercle. Sexual dimorphism in legs III and IV; femur, patella and tibia less enlarged, metatarsus III without enlarged spindle. Coloration pattern in ethanol differs in leg IV; femur, tibia and patella not reddish, but of the same color as the other legs.

Variation: Measurement variations in Table 1. Tubercles on mesotergal areas, free tergites and free sternites variable in size and number. Dimension of medial spiniform apophysis on free tergite III varies from one to two times the segment length. Coxae of male pedipalps vary in number of dorsoproximal mesal tubercles (2–3) and ventro-ectal (3–5–6) tubercles. Tarsus of pedipalps with variable number of setiferous tubercles, eetal 4–5 setiferous tubercles, mesal 4–5 setiferous tubercles. Tarsal formula: 7:12–14:9:9.

Table 1.—*Heterolacurbs perezassoi* new species: Dorsal scutum, pedipalp and legs measurements expressed in millimeters.

♂ (holotype) CZACC 3.3179		♂ (paratype) CZACC 3.3180	♂ (paratype) CZACC 3.3182	♀ (paratype) CZACC 3.3181	♀ (paratype) CZACC 3.3183
Dorsal scutum					
DSL	2.32	2.40	2.44	2.48	2.40
DSW	2.24	2.28	2.24	2.24	2.08
PL	0.96	0.96	1.00	0.92	1.00
PW	2.04	2.08	2.04	1.84	1.80
Pedipalp					
Tr	0.43	0.45	0.43	0.38	0.38
Fe	2.20	2.10	2.13	1.95	1.88
Pa	1.25	1.25	1.20	1.23	1.18
Ti	0.80	0.78	0.80	0.75	0.78
Ta	1.53	1.55	1.55	1.43	1.45
Total	6.21	6.13	6.11	5.74	5.67
Leg I					
Tr	0.35	0.40	0.35	0.35	0.30
Fe	2.00	2.00	2.00	1.85	1.85
Pa	0.55	0.50	0.55	0.45	0.50
Ti	1.80	1.75	1.75	1.60	1.55
Mt	2.75	2.62	2.55	2.35	2.35
Ta	1.25	1.20	1.20	1.05	1.10
Total	8.70	8.47	8.40	7.65	7.65
Leg II					
Tr	0.50	0.55	0.50	0.40	0.45
Fe	4.90	4.75	4.55	4.55	4.70
Pa	0.75	0.80	0.80	0.70	0.75
Ti	4.10	4.00	3.85	3.80	3.80
Mt	5.70	5.04	5.10	4.80	4.80
Ta	3.02	3.05	2.85	2.80	2.80
Total	18.97	18.19	17.65	17.05	17.30
Leg III					
Tr	0.55	0.55	0.60	0.50	0.55
Fe	3.50	3.35	3.30	3.25	3.15
Pa	0.90	0.92	0.90	0.80	0.80
Ti	2.25	2.30	2.25	2.15	2.15
Mt	4.20	3.80	3.95	3.60	3.60
Ta	1.75	1.65	1.70	1.60	1.50
Total	13.15	12.57	12.70	11.90	11.75
Leg IV					
Tr	0.60	0.65	0.65	0.60	0.55
Fe	4.60	4.35	4.40	4.65	4.75
Pa	1.05	0.95	1.00	0.90	0.95
Ti	2.95	2.75	2.75	2.60	2.90
Mt	5.58	5.16	5.28	4.92	4.98
Ta	2.25	2.15	2.10	1.95	1.80
Total	17.03	16.01	16.18	15.62	15.93

Distribution.—Known from Puerto Rico: Sierra de Guardarraya, Barrio Los Pollos (Fig. 10).

Natural history.—The specimens were collected in the backyard of a house (170 m a.s.l.), under damp logs (see Armas 2010:59, fig. 3E), living together with species of the family Cosmetidae and other unidentified opilions, also sharing the habitat with the buthid scorpion *Tityus obtusus* (Karsch 1879), undetermined Corinnidae, *Avicularia* sp. (Theraphosidae) and the whip spider *Phrynus longipes* (Pocock 1894) (Armas 2010).

Remarks.—In *H. perezassoi* n. sp. the dorsodistal spine on femur II–IV of the legs is absent, but in *H. ovalis* it is present. This remarkable interspecific difference also occurs between the Cuban stenostygnids *Galibrotus carlotanus* Šilhavý 1973, *G. riedeli* Šilhavý 1973 and *G. matiasis* Avram 1977. This spine is present in the first species, but lacking in the last two. The absenee or presence of this character in different species from the same genus seems to occur indistinctly. The length of legs I to IV in *H. ovalis* varies from 9.0, 18.0, 14.5, 18.0 mm in the holotype (Roewer 1912) to 5.0, 10.4, 7.4, 10.6 in the holotype of *Martibianta virginsulana* (Šilhavý 1973). However, among the specimens of *Heterolacurbs perezassoi* examined there is no remarkable variability in the length of the legs (see Table 1); they exhibit measurements very similar to Roewer's type.

Regarding the coloration pattern, even when both species have a lighter medial region, the new species does not exhibit lighter spots on each tubercle of the mesotergal areas and posterior margin.

Manahunca silhavyi Avram 1977 and *H. perezassoi* share similarities such as long, smooth legs; long pedipalps and pattern of coloration. However, the armature of the dorsal scutum and free tergites are very different: in *M. silhavyi*, they are unarmed and only provided with small tubercles.

Before this study, the only known records for Stenostygninae in the Antilles were from Haiti on the island of Hispaniola (2 genera and 2 species), St. Johns and St. James, United States Virgin Islands (1 genus and 1 species), and from Cuba (5 genera and 13 species). *Heterolacurbs perezassoi*, from the island of Puerto Rico constitutes a new distributional record of this subfamily. However, with the complicated geological history of the Antilles and favorable conditions for harvestmen (tropical forests, mountainous territories and high humidity), we expect to find new members of this subfamily in this area. The diversity of stenostygnid species found in Cuba shows that every island of the Greater Antilles could have experienced a high level of speciation, which is why we highly recommend more collecting of these Laniatores around the Antillean region.

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The identity of *Hadrobunus grandis*: reassignment of *Leiobunum aurugineum* to *H. grandis* and *H. nonsacculatus* new species (Opiliones: Sclerosomatidae: Leiobuninae)

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Abstract. Even though *Hadrobunus grandis* (Say 1821) is the type species of *Hadrobunus*, its identity has been uncertain since its original description. The type specimens were collected in coastal Georgia and/or northeastern Florida during the winter of 1817–1818, not from the mid-Atlantic Region (e.g.,Virginia, Maryland) as assumed by some authors. This error has resulted in persistent confusion with *H. maculosus* (Wood 1868), the dominant *Hadrobunus* species in the mid-Atlantic region. The type specimens of *H. grandis* were lost or destroyed, but all surviving evidence suggests that the species known as *Leiobunum aurugineum* Crosby & Bishop 1924 is a synonym of *H. grandis*. Examination of available museum specimens revealed two species. Populations east of the Apalachicola River correspond to the historical description of *L. aurugineum* in having sacculate penes, and are thus identical to *H. grandis*; those west of the river lack penial sacs and are placed in the new species *H. nonsacculatus*.

Keywords: Harvestmen, taxonomy, North America

The recent discovery of new species of the endemic North American genus *Hadrobunus* Banks 1900 (Shultz 2010) highlights the need to resolve a long-standing uncertainty about the identity of the type species *Hadrobunus grandis* (Say 1821). Thomas Say based his description of *Phalangium grandis* on specimens collected during an expedition by the Academy of Natural Sciences of Philadelphia to northeastern Florida and the coastal islands of Georgia (Fig. 1) that lasted from December 1817 to April 1818 (Bennett 2002). The type specimens were not illustrated and were soon lost or destroyed (LeConte 1859; Weiss & Zeigler 1931). Therefore, Say's (1821:67–68) terse description is critical to identifying *H. grandis* and is reprinted here, with the current author's clarifications in brackets.

> *P. grandis*. Body oval, covered with short spines; ocular tubercle spinous; feet rather short. Inhabits the Southern States... *Body* oblong-oval, scabrous [hard and rough, scab-like], with approximated [closely spaced], robust, short, acute spinules; rufo-ferugineous [sic] [reddish brown; color of iron rust], two impressed transverse lines before the middle [demarcations of meso- and metapeltidia]; *ocular tubercle* prominent, slightly contracted at base, crowned with numerous, robust, acute spinules; *clypeus* hardly elevated; *feet* rather short; *pectus* [coxae] with numerous, minute, acute granules; *venter* with but few. Length, female nearly seven-twentieths of an inch [~ 9 mm]. Much the largest I have seen.

The locality, cuticular armature, color and body size correspond uniquely to *Leiobunum aurugineum* Crosby & Bishop 1924. Consequently, I propose *L. aurugineum* as a junior synonym of *Hadrobunus grandis*. Significantly, results from recent molecule-based phylogenetic analysis (Hedin et al. 2012; Burns et al. 2012) show that *L. aurugineum* is more closely related to *Hadrobunus maculosus* and *Leiobunum formosum* (soon to be transferred to *Hadrobunus*: J.W. Shultz unpublished data) than to other *Leiobunum* species.

Summary of the *H. grandis* problem.—Say often used the vague term "the Southern States" in describing the distribution of specimens collected during his 1817–1818 expedition, and many subsequent researchers appear to have been unaware of the original collection locality of *Phalangium grandis*. Given Say's association with Philadelphia, the range of the species was widely thought to include such comparatively northern locales as Maryland and Virginia. Thus, when Wood (1868) described *Phalangium maculosum* (now *Hadrobunus maculosus*) from Pennsylvania and West Virginia without having seen *P. grandis* or making any association between the two species, the stage was set for more than a century of confusion.

For example, during a brief but aetive period (1887–1893), Weed published several treatments on the harvestman fauna of the northern midwestern states (summarized by Cokendolpher & Zeiders 2004) and his opinions on the taxonomy of *Phalangium grandis* and *P. maculosum* changed frequently, leading to the transfer of these and many other harvestman species to "*Liobunum*." Based on my own unpublished work, there appear to be four typical *Hadrobunus* species in the region: three are currently undescribed and one, *H. maculosus*, had been introduced to Illinois by 1883 (i.e., Livingston County: 1 ♂, Dwight, 41.0930°N, 88.4273°W, 8 August 1883, coll.?, Illinois Natural History Survey, Specimen Number 0006). Given the inadequate species descriptions of Say and Wood and persistent taxonomic emphasis on coloration as a diagnostic feature, it is understandable that Weed and others found it difficult to stabilize the concepts of *Phalangium maculosum* and *P. grandis*.

Banks (1900) erected the genus *Hadrobunus* to accommodate *Phalangium grandis* and *P. maculosum* and then added to the confusion by stating that *H. grandis* occurs in the "E. States" and *H. maculosus* occurs in the "S. States" (Banks 1901:677; repeated in Comstock 1912, 1968). This apparently led Crosby & Bishop (1924:21) to identify a *Hadrobunus* specimen from Richmond, Virginia as "*H. grande*" and specimens from southern Georgia as "*H. maculosum*." However, the specimen from Virginia was almost certainly *H. maculosus*, and the specimens from Georgia were most likely not *H. maculosus*, because this species reaches its southern limit in central North Carolina (J.W. Shultz unpublished observation). In her survey of Ohio harvestmen, Walker (1928) appeared to surrender to this confusion in stating that both species occur in "all counties," although it appears that neither does.

Bishop (1949) offered his own geographic criterion for distinguishing between the two species, which has been used by most subsequent researchers (e.g., Cokendolpher & Lee 1993). He considered *H. maculosus* to be a northern species that reaches its southern limit in Kentucky, Ohio, and West Virginia and *H. grandis* to be a southern species that occurs in the "southeast and ... [is] particularly abundant in the Atlantic coastal states" (Bishop 1949:214). These distributions correspond roughly to that of an undescribed species that dominates the Great Lakes Region (J.W. Shultz unpublished observation) and *H. maculosus*, respectively. Bishop's geographic demarcation implies that he had established morphological criteria to distinguish between two *Hadrobunus* species, even if they do not correspond to *H. maculosus* and *H. grandis*. But this does not appear to be the case. Following the strategy of most previous researchers, Bishop emphasized coloration in distinguishing among harvestman species.

> *Hadrobunus maculosus* differs from *H. grandis* in being generally lighter in color, in lacking conspicuous, sharp-pointed denticles on the dorsal surface of the body, in having the legs with a banded appearance rather than mottled or blotched, and in having more prominent rows of light spots on the dorsal surface of the abdomen (Bishop 1949: 216).

These criteria are problematic. In Maryland, for example, coloration in adult *H. maculosus* changes during late summer and autumn, with high contrast patterns in early summer (light-brown background, transverse rows of prominent spots, distinct banding on legs) and with progressive darkening of background and loss of contrast throughout the season (J.W. Shultz original observation). Thus early-season specimens correspond to Bishop's description of *H. maculosus* and late-season specimens correspond to his description of *H. grandis*. Furthermore, I have seen several of the specimens Bishop used in his 1949 treatment of *Hadrobunus*. Those "*H. maculosus*" from eastern New York (Albany Co.) are *H. maculosus*, those from central New York (Tompkins County) include both *H. maculosus* and the undescribed Great Lakes species, and those from Quicksand, Kentucky (which I have not seen) were almost certainly a second undescribed species that ranges from the northern Great Smoky Mountains north through the western Appalachians to the Ohio River (J.W. Shultz unpublished observation).

I conclude that past error and confusion has been so profound that, except for its initial description and those of its junior synonym *Leiobunum aurugineum*, all previous criteria aimed at diagnosing *Hadrobunus grandis* should be rejected. *Hadrobunus grandis*, which has heavy dorsal armature and a short sacculate penis (Fig. 2), occurs in the extreme southeastern United States (South Carolina, Georgia, Florida) (Fig. 1). Its distribution does not overlap or abut that of the poorly armed and nonsacculate (Fig. 8) *H. maculosus*, which is distributed along the eastern seaboard of the United States from central North Carolina to New Hampshire and has a westward limit that roughly corresponds to the Eastern and St. Lawrence Continental Divides (J.W. Shultz unpublished observations). The distribution of *H. maculosus* abuts or overlaps those of at least four undescribed *Hadrobunus* species, a situation that served to perpetuate confusion in distinguishing between *H. grandis* and *H. maculosus*.

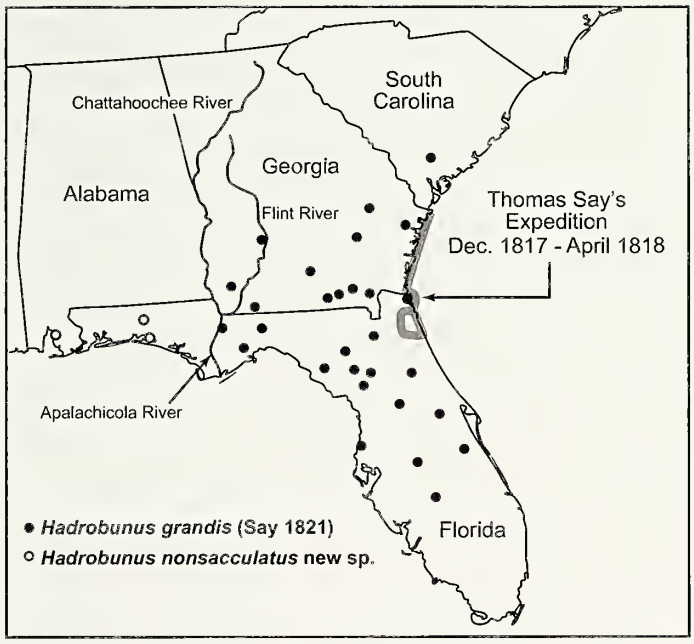


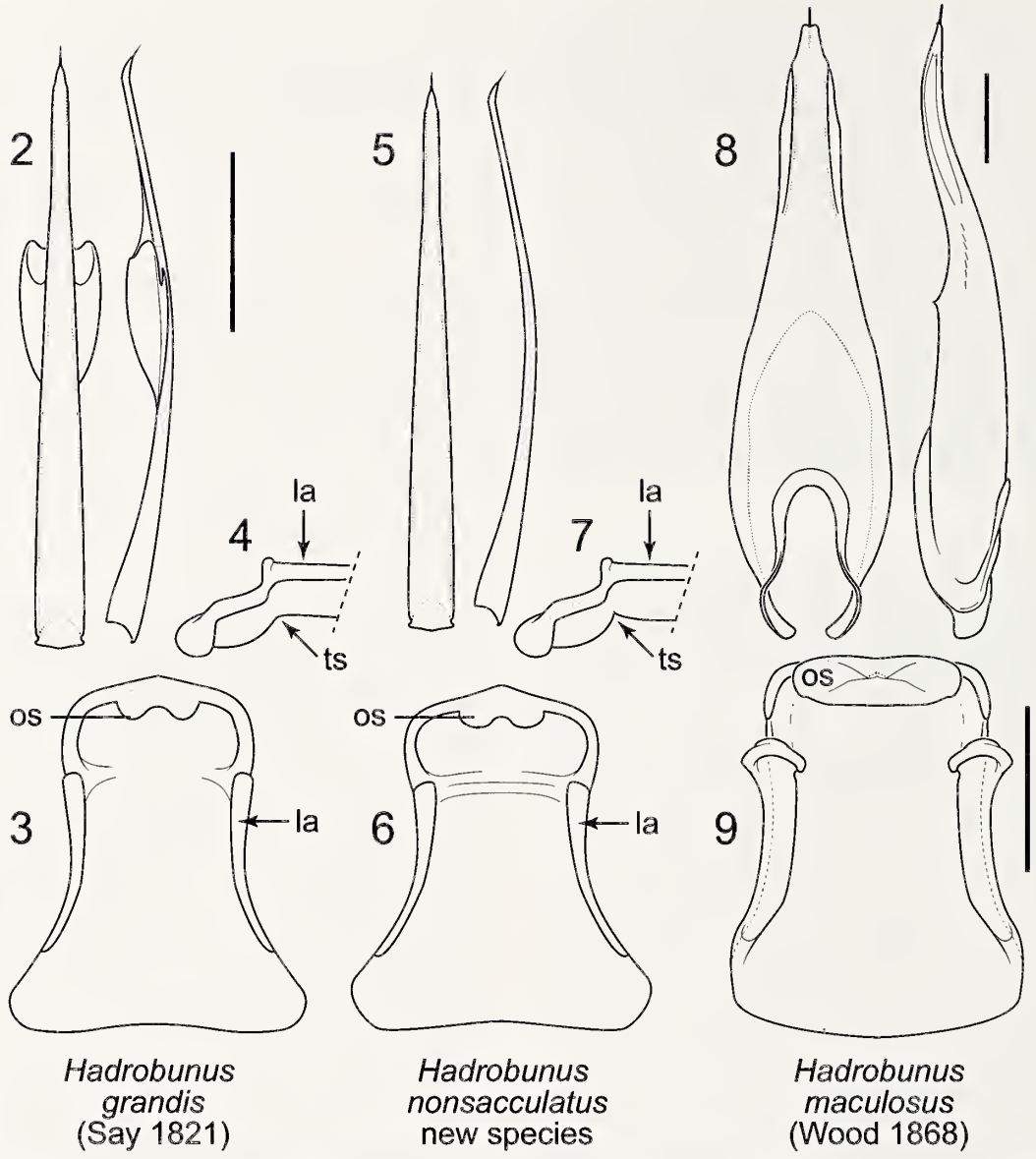
Figure 1.—Map of the southeastern United States showing collection localities of specimens used in the current study and the approximate route taken by Thomas Say during the 1817–1818 expedition by the Academy of Natural Sciences of Philadelphia.

A new species.—In attempting to establish the geographic range and morphological variation of *Hadrobunus grandis*, I found significant differences between populations separated by the Apalachicola River and its major western tributary, the Chattahoochee River (Fig. 1). Specifically, all male specimens obtained east of these rivers had sacculate penes (Fig. 2), and all male specimens west of the rivers lacked sacs (Fig. 5). Inspection of females suggested that the populations west of the Apalachicola River have a deep transverse sulcus spanning the genital operculum (Figs. 6, 7), while those east of the rivers have a shallow sulcus (Figs. 3, 4). Thus, the species historically known as *Leiobunum aurugineum* represents two species, *Hadrobunus grandis* east of the Appalachicola River and *H. nonsacculatus* west of that river. The river appears to be a major phylogeographic barrier in the southeastern United States (Soltis et al. 2006).

METHODS

I conducted all observations using a Leica MZ APO dissecting microscope (16× ocular, 0.63× objective, 8–80× zoom). Drawings were made with a drawing tube and then digitized and traced in Adobe Illustrator. Photographs were obtained with a PaxCam 3 digital camera mounted on a Wild Heerbrugg Makrozoom 1:5 with 6.3–32× objective. Images obtained at different focal planes were combined using Helicon Focus software (HeliconSoft, Kharkov, Ukraine).

Specimen repositories and abbreviations.—Specimens examined for this study were obtained from the following institutions: American Museum of Natural History, New York (AMNH); Field Museum of Natural History, Chicago (FMNH); Florida State Collection of Arthropods, Gainesville (FSCA); Illinois Natural History Survey, Champaign (INHS); Museum of Comparative Zoology, Harvard University (MCZ); Mississippi Entomological Museum, University of



Figures 2–9.—Genital structures of *Hadrobunus grandis*, *H. nonsacculatus* and *H. maculosus*. 2–4. *H. grandis*; 2. Penis in dorsal (on left) and lateral (on right) perspectives; 3. Dorsal view of inner surface of female genital operculum; 4. Lateral view of anterior portion of female genital operculum (semi-diagrammatic) showing shallow transverse sulcus (*ts*); 5–7. *H. nonsacculatus*; 5. Penis in dorsal (on left) and lateral (on right) perspectives; 6. Dorsal view of inner surface of female genital operculum showing transverse phragma connecting the anterior margins of levator muscle apodemes (*la*); 7. Lateral view of anterior portion of female genital operculum (semi-diagrammatic) showing deep transverse sulcus (*ts*). 8, 9. *H. maculosus*; 8. Penis in dorsal (on left) and lateral (on right) perspectives; 9. Dorsal view of inner surface of female genital operculum. Figs. 2–7 are depicted at the same scale. Scale bars = 1 mm.

Mississippi (MEM); National Museum of Natural History, Washington, D.C. (NMNH); Texas Memorial Museum, University of Texas, Austin (TMM); Museum of Texas Tech University, Lubbock (TTU); University of Maryland, College Park, author's collection (UMD).

TAXONOMY

Family Sclerosomatidae Simon 1879
Subfamily Leiobuninae Banks 1893
Hadrobunus Banks 1900

Hadrobunus Banks 1900:199.

Type species.—*Phalangium grandis* Say 1821, by original designation (Banks 1900). Banks erected *Hadrobunus* to accommodate *P. grandis* and *P. maculosum* Wood 1868, but he misidentified *P. maculosum* as *P. grandis*. I advocate retaining *Phalangium grandis,* as diagnosed here, as the type species for *Hadrobunus*. As detailed above, early descriptions of the two species were too superficial to allow them to be reliably distinguished, so retaining *P. grandis* as the type species introduces no complications and stabilizes the literature.

Diagnosis.—Anterior margin of female genital operculum with median sclerotized lobe or sclerite (Figs. 3, 6, 9). Coxa II with conical spike with accessory lateral point located near retrolateral articulation with trochanter (i.e., retrolateral coxal spur II) (Fig. 12). Scutum of both sexes with variably expressed sharp, posteriorly-curved (retrorse) tubercles. Ventral surface of palpal tibia with sexually dimorphic armature:

male with field of small, blunt-tipped tubercles, female with sharp, conical, distally slanted denticles. Prolateral rows of denticles present on all pedal coxae; retrolateral rows of denticles present on all pedal coxae except leg III. Legs of female (and usually male) relatively short: length of femur I subequal to body length or shorter. Pedal femora of both sexes without pseudoarticulations or nodules, tibiae without pseudoarticulations. Surfaces of pedal coxae tuberculate. Ocularium domelike, not constricted at base, not canaliculate, each carina usually with a row of 5 to 8 denticles.

Hadrobunus grandis (Say 1821)
Figs. 2–4

Phalangium grandis Say 1821:67; Say 1859:14 ["Southern States" = coastal Georgia and northeastern Florida].
Phalangium grande Say: Wood 1868:34; Underwood 1885:168.
Phalangium (?) *grande* Say: Weed 1889a:105.
Liobunum grande (Say): Weed 1892a:192–193 [Illinois, Ohio: misidentifications; Virginia, District of Columbia: misidentifications, *H. maculosus*]; Banks 1911:456 [North Carolina: Swannanoa Valley, misidentification, *H. fusiformis*?].
Liobunum similis Weed 1890:918 [Ohio: misidentification]; Cokendolpher & Zeiders 2004:9.
Liobunum grande variant *similis*: Weed 1892a:193, plate 9, Figs. 1–2g [Ohio: misidentification]; Roewer 1910:255.
Astrobunus (?) *grande* (Say): Weed 1890:917.
Leptobunus grande (Say): Banks 1893:209–210.
Hadrobunus grande (Say): Banks 1900:199; Banks 1901:677; Banks 1904:256 [misidentification of *H. maculosus*].
Hadrobunus grandis (Say): Roewer 1910:254–255 [USA: Illinois, Ohio, Virginia: misidentifications]; Roewer 1923:919 [British Columbia: locality incorrect; see also Cokendolpher & Lee 1993; Bragg & Holmberg 2009]; Walker 1928:168, fig. 24 [Ohio: misidentification]; Crosby, Wolf & Bishop 1928:1076 [New York: misidentification of *H. maculosus*]; Muma 1944:24 [Maryland: misidentification of *H. maculosus*]; Edgar 1966:353, 359, Edgar 1990:568 [description incorrect].
Leiobunum aurugineum Crosby & Bishop 1924:13–14, pl. 2, fig. 8; Davis 1934:664–666, fig. 2; Edgar 1990:574,578 [East of Apalachicola and Chattahoochee Rivers]. NEW SYNONYMY.

Type material examined.—*Leiobunum aurugineum* Crosby & Bishop 1924. Holotype male, USA: *Georgia*: Charleton County: Okefenokee Swamp, Billy's Island, 30.8052°N, 82.3404°W, [?] June 1912, coll.? (AMNH).

Other material examined.—USA: *Florida*: Alachua County: 1 ♂, Gainesville, Live Oak Hammock, 26.6516°N, 82.3248°W, 22 July 1942, coll.? (INHS: 00075); many ♂ and ♀, same locality, 4 September 1929, N.W. Davis (AMNH), 1 ♀; same locality, 27 June 1969, D.L. Brown (TTU Z-58,743); 1 ♀, Newberry, 29.6463°N, 82.6065°W, 19 April 1930, T.H. Hubbell (AMNH); Bradford County: 1 ♂, 1 ♀, near Starke, 29.9441°N, 82.1098°W, November–December 1943, H.S. Dybas (FMNH). Columbia County: 2 ♂, Santa Fe River, 29.8478°N, 82.6913°W, 29 October 1929, T.H. Hubbell (AMNH). Dixie County: many ♂ and ♀, no specific locality [county center used for coordinates], 29.6516°N, 83.1649°W, date?, coll.? (AMNH). Glades County: 1 ♀, Fisheating Creek, ~15 mi [~24 km] NW Moore Haven, 5 mi [8 km] E Palmdale, in rotten log, 27.1731°N, 81.4632°W, 28 August 1963, K.J. Stone (FSCA). Hernando County: 1 ♂, 9 ♀, Withlacooche State Forest, McKethan Lake State Park, mesic to dry woodland, 27.6648°N, 81.5157°W, 17–19 September 1982, G.B. Edwards (FSCA); 1 ♂, 2 ♀, Weeki Wachee, off Hwy 50, Boy Scout Reservation, sand hill S power line, 28.5155°N, 82.5729°W, 18 September 1987, D. Corey (TTU Z-58,827). Lake County: 3 ♀, no specific locality [county center used for coordinates], 28.7028°N, 81.7787°W, 17 May 1982, W.W. Smith (FSCA). Leon County: 2 ♀, 5 mi [8 km] N Tallahassee, under pine log, 30.5498°N, 84.2823°W, 14 June 1982, W.H. Cross (MEM). Levy County: 1 ♂, Williston, 29.3875°N, 82.4468°W, 30 May 1981, L. O'Berry (FSCA). Liberty County: 1 ♂, Torreya State Park, 30.33°N, 84.47°W, 18 December 1967, W. Ivie (AMNH). Marion County: 5 ♂, 5 ♀, Belleview, under log, 29.0552°N, 82.0623°W, 20 May 1960, H.A. Denmark (FSCA). Nassau County: 1 ♀, Fort Clinch State Park, edge of oak forest in old dunes, 30.6997°N, 81.4444°W, 14 September 1958, T.J. Walker (FSCA). Orange County: 1 ♂, Orlando, University of Central Florida Campus, 28.5378°N, 81.3775°W, [?]1983, D.T. Corey (NMNH). Putnam County: Ordway Preserve, night, 27.6648°N, 81.5157°W; 1 ♀, 25 October 1983, G.B. Edwards, M.K. Stowe (FSCA); 1 ♂, 1 ♀, same locality, 30 August 1984, G.B. Edwards, M.K. Stowe (FSCA). Wakulla County: 1 ♀, St. Mark's National Wildlife Refuge, 1 mi [1.61 km] SW Panacea, 30.0518°N, 84.4068°W, 21 June 1979, C.R. Smith (FSCA).

Georgia: Bacon County: 1 ♀, no specific locality [county center used for coordinates], 31.5412°N, 82.4319°W, 2 October 1929, T.H. Hubbell (AMNH). Berrien County: 1 ♀, Nashville, 31.2074°N, 83.2502°W, 10 June 1955, H.S. Dybas (FMNH). Bulloch County: 1 ♀, 6 mi [9.65 km] S Statesboro, sphagnum bog, 32.3277°N, 81.7781°W, 12–13 October 1984, G.B. Edwards, L.S. Vincent (FSCA). Charlton County: 2 ♂, Mixon's Hammock, Okefenokee Swamp, 30.8183°N, 82.3918°W, 16 June 1912, coll.? (AMNH); many ♂ and ♀, no specific locality [county center used for coordinates], 30.7917°N, 82.0843°W, 29 September 1929, T.H. Hubbell (AMNH). Decatur County: 2 ♀, Eldorendo, 31.0441°N, 84.6519°W, 12 September 1929, T.H. Hubbell (AMNH); many ♂ and ♀, Faceville, 30.7532°N, 84.6399°W, 12 September 1929, T.H. Hubbell (AMNH). Lanier County: 1 ♂, 1 ♀, Stockton, 30.9376°N, 83.0072°W, 1 November 1929, N.W. Davis (AMNH). Liberty County: 3 ♂, 3 ♀, Midway, 31.8050°N, 81.4307°W, 3 October 1930, T.H. Hubbell (AMNH). Lowndes County: 1 ♂, Valdosta, 30.8327°N, 83.2784°W, 26 October 1929, N.W. Davis (AMNH). Sumter County: many ♂ and ♀, Maddox, 31.9969°N, 84.24225°W, 1 October 1929, T.H. Hubbell (AMNH).

South Carolina: Colleton County: 4 ♂, 1 ♀, no specific locality [county center used for coordinates], 33.0399°N, 80.8823°W, 26 September 1930, T.H. Hubbell (AMNH).

Diagnosis.—*Adult males and females:* Scutum distinctly convex (more so in female); mesopeltidium, metapeltidium, scutum and free tergites densely armed with robust retrorse tubercles; dorsal cuticle well sclerotized, thick and hard. Body rusty brown to yellow-orange; lighter ventrally. *Male:* Penis (Fig. 2) with pair of large, thin-walled sacs occupying about 25% penis length; sacs positioned usually far from glans-shaft joint, specifically, anterior margin of sac separated from joint by 20–25% penis length. *Female:* Genital operculum flexed ventrally (Fig. 4) with transverse bend at anterior margin of

apodemes of levator muscles (Fig. 3), but not forming deep transverse cleft as in female *H. nonsacculatus* (Figs. 6, 7). Otherwise uniquely similar to female *H. nonsacculatus*: ventral surface somewhat inflated anterior to transverse flexure (Figs. 4, 7); opercular sclerite (Figs. 3, 6) occupying median half of anterior lip, with prominent median notch.

Description.—For descriptions see *Leiobunum aurugineum* in Crosby & Bishop (1924) and Davis (1934).

Distribution.—Extreme southeastern United States, including Florida, southern and eastern Georgia, southeastern South Carolina; western limit appears to correspond to Apalaehicola and Chattahoochee Rivers (Fig. 1).

Hadrobunus nonsacculatus new species
Figs. 5–7

Leiobunum aurugineum Crosby & Bishop 1924:13–14, pl. 2, fig. 8; Davis 1934:664–666, Fig. 2; Edgar 1990:574, 578 [in part, specimens from Coastal Alabama and coastal Florida west of Apalachicola River].

Type material.—Holotype male, USA: *Florida*: Okaloosa County: Fred Gannon Rocky Bayou State Park, 30.4965°N, 86.4292°W, [?] June 1983, L. Robbins (TTU-Z 58,740). Paratype: ♀, same data as holotype (TTU-Z 58,742).

Other material examined.—*Alabama:* Baldwin County: 2 ♂, Daphne, on beach, 30.6061°N, 87.9126°W, 19 July 1931, Dietrich (AMNH).

Florida: Okaloosa County: 1 ♂, near Delaco, 30.7448°N, 86.5954°W, 11–12 August 1935, T.H. Hubbell (MCZ 37083); 2 ♂, 1 ♀, Fred Gannon Rocky Bayou State Park, 30.4965°N, 86.4292°W, [?] June 1983, L. Robbins (TTU-Z 58,738; 58,741; 58,742).

Diagnosis.—Essentially identical to *Hadrobunus grandis* except for the following: *Male*: Penis without sacs (Fig. 5). *Female*: Transverse anterior sulcus of genital operculum a deep cleft (Fig. 7: *ts*), expressed internally as a low, transverse phragma appearing to connect the anterior ends of the apodemes of the levator muscles (Fig. 6).

Description of male holotype.—Measurements in mm. Male holotype: body length 6.14. Palp: femur 1.56, patella 0.72, tibia 1.05, tarsus 1.57. Leg I: femur 4.87, patella 1.23, tibia 3.93, basitarsus 4.50, telotarsus 6.63. Leg II: femur 7.75, patella 1.51, tibia 6.28, basitarsus 6.37, telotarsus 14.68. Leg III: femur 5.20, patella 1.43, tibia 3.40, basitarsus 3.31, telotarsus 6.54. Leg IV: femur 7.41, patella 1.54, tibia 4.97, basitarsus 6.03, telotarsus 8.39. Penis: 3.98.

Dorsum (Fig. 10): Carapace unarmed near ocularium but with scattered conical-to-retrorse denticles on submarginal surfaces. Median preocular margin slightly elevated with three imperfect rows of sharp conical denticles (one median, two lateral) extending about half way to ocularium. Ozophore slightly elevated. Ocularium well armed with six conical denticles surrounding each lens, an additional four denticles on anterior surface and single lateral denticle on right side behind lens; a few scattered erect setae. Supracheliceral lamina well developed; thin laterally but with bilateral pair of prominent, closely spaced anterior processes; each process bearing small denticles, concentrated terminally. Meso- and metapeltidia with dense covering of robust tubercles, most terminating in dark spinules; metapeltidial tubercles retrorse. Scutum convex, heavily sclerotized, with dense coat of robust, retrorse tubercles, most tubercles with posteriorly projecting dark spinule. Scutum with five tergites. Three free tergites armed like scutum, but tubercles decreasing in size and density posteriorly. Anal operculum with a few small, simple tubercles. Dorsum without setae.

Venter. Genital operculum with rebordered anterior margin, anterior median portion protruding slightly; operculum armed with submarginal rows of well-developed flat-topped to weakly tricuspid denticles; surface with small scattered tubercles and erect macrosetae. Sternites with low tubercles medially, tubercles more pronounced laterally; pleurosternites present. Labrum with bilateral pair of distal tubercles.

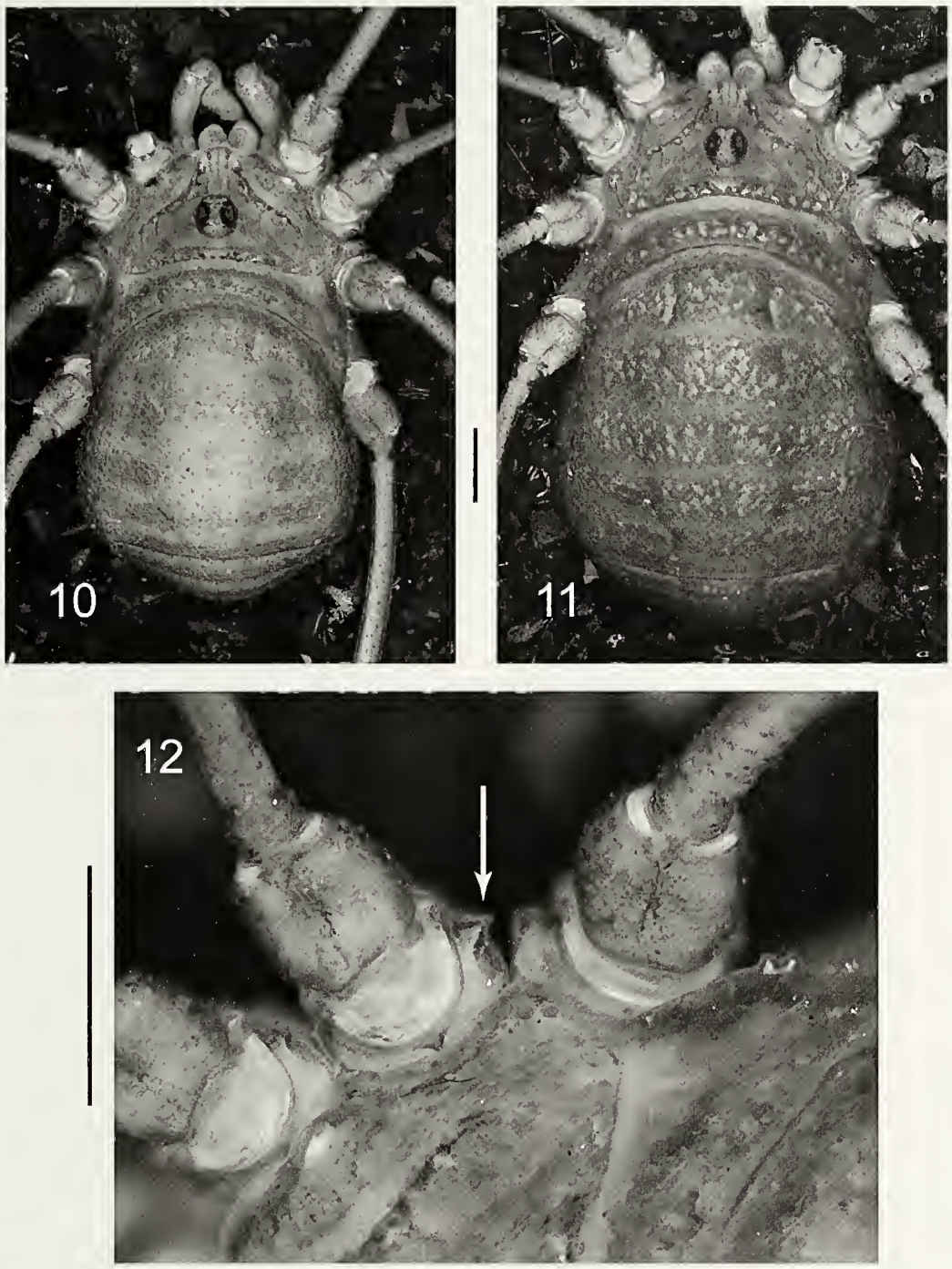
Appendages. Chelicera: Unremarkable. Basal article with proximoventral triangular apophysis and imperfect proventral row of macrosetae; second article with dorsal field of erect macrosetae, prolateral surface with field of sort macrosetae increasing in density toward base of fixed cheliceral digit, small tubercle present at base of fixed finger.

Palps: Femur with long retrolateral row and distodorsal-to-retrolateral field of thorn-like denticles and ereet macrosetae; prolateral surface largely unarmed except for long row of blunt denticles and erect macrosetae; prodistal margin with two large, thornlike denticles. Patella armed with scattered thorn-like denticles and erect macrosetae, but retrodistal surface largely unarmed; prodistal apophysis undeveloped, but indicated by tuft of macrosetae. Tibia with field of scattered peg-like denticles proximoventrally; three denticles on retroventral distal margin, proventral distal margin unarmed; two small denticles on proximodorsal surface; erect macrosetae scattered on all surfaces although substantially reduced on prolateral surface; fine distally recumbent setae on prolateral surface. Tarsus with dense coat of fine, distally recumbent microsetae and erect macrosetae; proventral surface with long row of dark peg-like denticles; retroventral surface with short proximal row of small, sharp denticles. Claw with a short proximal row of three teeth ventrally.

Legs: Coxae with numerous low tubercles, each coxa with long prolateral row of prominent, flat-topped to weakly tricusped denticles; all but coxa III with similar retrolateral row of denticles. Coxa II with retrolateral spur (Fig. 12) in form of sharp conical denticle with accessory lateral cusp, similar but smaller retrolateral spur on coxa I. Prolateral surface of coxa III opposite retrolateral spur II protuberant (Fig. 12). Trochanters with small thorn-like denticles on pro- and retrolateral surfaces. Distal leg articles unremarkable.

Penis. Dorsoventrally flattened, tapering gradually toward tip, glans-shaft joint indicated by slight constriction; no sacs or alae (Fig. 5).

Coloration. Body a general orange-brown (Fig. 10). Ocularium with light median stripe. Surface of carapace lightly mottled by darker and lighter sigillary markings. A bilateral pair of dark lines punctuated by light spots begins anteriorly on either side of preocular region and passes posterolaterally, terminating laterally; a similar color pattern on meso- and metapeltidia. Scutum with segmentation reflected in alternating transverse bands of slightly darker tergal regions and slightly lighter intertergal regions; median mark subobsolete, limited largely to slight median darkening on scutal tergite 1. Venter lighter than dorsum but anterior and posterior sternal



Figures 10–12.—*Hadrobunus nonsacculatus*. 10. Male, dorsal perspective. 11. Female, dorsal perspective. 12. Male, dorsal perspective highlighting retrolateral spur of coxa II as indicated by arrow. Figs. 10 and 11 are depicted at the same scale. Scale bars = 1 mm.

margins slightly darkened. Pedal coxae and trochanters essentialy concolorous with venter or slightly darker, but legs becoming lighter distally; tarsi yellow-brown. Coloration of palps similar to that of legs. Chelicerae light yellow-brown, except for darker sigillary markings.

Description of female paratype.—Measurements in mm: body length: 7.48. Palp: femur 1.26, patella 0.61, tibia 0.92, tarsus 1.64. Leg I: femur 4.76, patella 1.07, tibia 3.50, basitarsus 5.79, telotarsus 6.21. Leg II: femur 7.66, patella 1.27, tibia 5.84, basitarsus 6.14, telotarsus 11.03. Leg III: femur 4.65, patella 1.39, tibia 3.42, basitarsus 4.58, telotarsus 5.84. Leg IV: femur 7.28, patella 1.65, tibia 4.68, basitarsus 7.62, telotarsus 7.77.

As in the male, except of the following: *Venter*: Genital operculum with wide anterior lip, median portion protruding anteriorly; anterior portion flexing ventrally in lateral perspective at distinct transverse sulcus (Fig. 7), sulcus expressed internally as transverse phragma (Fig. 6); portion of operculum anterior to sulcus slightly inflated (Fig. 7); inner margin of anterior lip with pronounced sclerite projecting posteriorly, posterior margin of sclerite with broad median notch (Fig. 6). Labrum smooth, simple. *Appendages:* Chelicera: With fewer

setae than male. Palp: Femur less well-armed on the distodorsal and retrolateral surfaces, but retroventral row of denticles well developed; blunt prolateral denticles arranged in long proventral row, not prolateral row of the male. Patella with prodistal apophysis slightly developed. Tibia armed with sharp, distally slanted denticles on ventral and prolateral surfaces; no peg-like denticles. Tarsus unarmed, without pro- and retroventral rows of denticles. *Coloration*: Meso- and metapeltidium with more pronounced pattern of light dots against dark background (Fig. 11). Scutal and free tergites with numerous light dots and elongated markings, scutal tergites separated by lighter transverse bands; median dorsal figure expressed by darkened outline in scutal terga (and light lateral outline anteriorly).

Distribution.—Coastal Florida west of Apalachicola River and southern Alabama; western and northern limits unknown. *Hadrobunus grandis* and *H. nonsacculatus* appear to be separated by the Apalachicola River (Fig. 1).

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Comparative study of walking and climbing speeds among Neotropical harvestmen from Costa Rica

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Abstract. Relatively little is known about factors that contribute to microhabitat selection among Central American harvestmen. In this study, we compared walking and climbing speeds for five common species from Costa Rica representing the families Cosmetidae (3 species), Gonyleptidae (1 species) and Sclerosomatidae (1 species). Our sample included two arboreal species, two species that infrequently occupy perches in the vegetation, and one species that rarely climbs. Our analyses revealed no significant interspecific differences in climbing speed, although species with relatively long legs walked significantly faster than species with shorter legs. An arboreal habit did not correlate with increased climbing speed, and all species walked significantly faster than they climbed.

Keywords: Cosmetidae, Gonyleptidae, habitat selection, Opiliones, Sclerosomatidae

The kinematics of terrestrial and arboreal locomotion for members of the order Opiliones are poorly known (Sensenig & Shultz 2007), although individuals of most species of harvestmen generally walk in a manner similar to insects (Shultz & Pinto-da-Rocha 2007). Legs I, III and IV of the harvestman support the body and move in an alternating tripod gait, whereas leg II is used primarily as a tactile sensory organ during walking (Shultz & Pinto-da-Rocha 2007). In climbing, sclerosomatid harvestmen (*Leiobunum* spp.) use the large number of tarsomeres on leg II to form coils that enable individuals to cling to vegetation (Guffey et al. 2000).

Neotropical harvestmen occupy a variety of terrestrial and arboreal habitats including caves, trees, leaf litter, phytotelmata (tree holes and tank bromeliads), palm frond sheaths, rotting logs and cavities beneath rocks (Burns et al. 2007; Curtis & Machado 2007). Individuals may use spaces within vegetation or under rocks or logs as diurnal refugia (Acosta & Machado 2007; Proud et al. 2011). After dusk, however, harvestmen generally forage in the leaf litter and may also climb on vegetation in search of prey or mates (Machado et al. 2000; Willemart & Gnaspini 2004; Wade et al. 2011). Abiotic factors such as temperature and humidity have a major effect upon microhabitat selection and activity patterns (Todd 1949; Edgar 1971). Prior research has suggested a correlation between leg structure and the occupation of arboreal habitats (Curtis & Machado 2007). In Neotropical gonyleptid and temperate sclerosomatid harvestmen, individuals of species that have a greater number of tarsal segments or relatively longer legs tend to occupy higher perches in the understory and on tree trunks than others that possess fewer tarsomeres or shorter legs (Curtis & Machado 2007). Arboreal arachnid species with suspensory locomotion are predicted to benefit from pendulum mechanics and are predicted to possess relatively longer legs that confer greater capabilities than terrestrial species (Moya-Laraño et al. 2008).

Most ecological studies of Neotropical harvestmen are of South American species (Pinto-da-Rocha et al. 2005; Bragagnolo et al. 2007). Relatively little is known about the behavior, ecology or natural history of most species of harvestmen from Central America (Townsend et al. 2010; Townsend et al. 2011; Proud et al. 2012). The wet tropical forest at La Selva Biological Station, Costa Rica, supports a diverse harvestmen fauna including representatives of the families Cosmetidae (19 species), Gonyleptidae (3 species), Sclerosomatidae (6 species), Stygnommatidae (1 species), and Zalmoxidae (7 species), as well as the genus *Costabrimma* (2 species) (Proud et al. 2012). The most common species are *Cynorta marginalis* Banks 1909 (Cosmetidae), which actively climbs trees after dusk, and members of the genus *Prionostemma* (Sclerosomatidae), which frequently form loose aggregations on trees during the day and actively wander through the leaf litter as solitary individuals after dark (Wade et al. 2011). Other relatively common, but generally less abundant, species include the cosmetid harvestmen *Eupoecilaema magnum* Roewer 1933 and *Paecilaema* sp., which infrequently climb the vegetation but are most frequently encountered within palm fronds or decaying logs (Proud et al. 2012). Species representing the families Gonyleptidae, Stygnommatidae and Zalmoxidae inhabit the leaf litter and are rarely observed climbing vegetation (Proud et al. 2012).

The objective of this study was to examine the relationship between field observations of habitat use and locomotion in Costa Rican harvestmen. Specifically, we sought to compare walking and climbing speeds for five species of harvestmen: two highly arboreal (*C. marginalis* and *Prionostemma* sp.), two that are occasionally found on vegetation (*E. magnum* and *Paecilaema* sp.), and one primarily terrestrial species (*Glysterus* sp.). We predicted that the arboreal species would climb significantly faster than terrestrial species. Based upon general observations of the importance of relative leg length (reviewed by Curtis & Machado 2007), we also predicted that species with relatively longer legs (*Prionostemma* sp. and *E. magnum*) would walk and climb significantly faster than species with shorter legs (*C. marginalis* and *Glysterus* sp.).

METHODS

Our study was conducted at La Selva Biological Field Station (10°26′15.03″N, 84°00′1.19″W, datum: WGS84) from 6–19 July 2010. We captured harvestmen by hand opportunistically between 1200–1600 h from palm frond sheaths, tree trunks and leaf litter. Prior to testing, individuals were

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communally housed in mesh cylindrical containers (height = 23 cm, diameter = 14 cm) with damp paper towels. Small branches and leaf litter were added to the containers to provide structure and hiding plaees. Harvestmen were kept in a shade tent at ambient temperatures (25–30 °C) for 24–48 h prior to testing and were given fresh tropical fruit and water ad libitum.

We conducted trials from 1900–0100 h under red light to minimize disturbance of individuals (Hoenen & Gnaspini 1999). Trials were randomized with respect to type (walking or climbing), species and individual. To control for the impact of leg autotomy (a defense mechanism employed by *Prionostemma*, but not by the other species in our study: Gnaspini & Hara 2007) in both experiments, we only used adult harvestmen that had all eight legs (n = 30 individuals per species for all species except *Glysterus* sp., for which n = 24). The speed (cm/s) of each harvestman was measured once for walking and once for climbing in random sequence.

To determine a walking speed, we constructed a horizontal track (70 cm length × 8 cm wide) using four meter sticks. On each side of the track, the first meter stick was placed flat and a second was positioned on its side on top of the first, enabling us to easily read cm inerements while providing enough clearance (3.5 cm) for even the largest individuals. The floor of the trackway was lined with moistened paper towels. To prevent harvestmen from escaping, we placed thin sections of plexiglass over the top of the trackway. Similar to the protocol employed by Guffey (1999), we held each harvestman by legs III and IV at the 0 cm mark at the beginning of each trial until it was motionless. After gently releasing it, we measured the time that it took to walk to the 70 cm mark. In many trials, the harvestmen paused or ceased moving and then resumed walking. To determine speed while moving, we stopped timing during such pauses.

To determine the climbing velocity of each individual, we used a large, vertically suspended leaf (approximately 80 em in length) that was freshly cut from a split-leaf palm (*Geonoma cuneata*) as a test surface. Each night we used a new, freshly cut leaf. We marked each leaf with 10 cm increments to a final mark of 70 cm and suspended it in the vertical position. We used the same protocol for handling and observing harvestmen as in the walking experiment. After measuring walking and climbing speeds, we preserved individuals in 70% ethanol.

The residuals for the walking data were not normally distributed (Shapiro-Wilk test: W = 0.891, P < 0.001). Residuals for climbing velocities likewise did not meet the assumptions of normality (Shapiro-Wilk test: W = 0.925, P < 0.001). Therefore we applied a log(x+1) transformation to the data. Log transformed walking speeds met the assumptions of normality (Shapiro-Wilk test: W = 0.984, P = 0.08) and homogeneity (Bartlett test: K^2 = 2.128, df = 4, P = 0.712). Log transformed climbing speeds also met the assumptions of normality (Shapiro-Wilk test: W = 0.986, P = 0.248) and homogeneity (Bartlett test: K^2 = 4.455, df = 4, P = 0.348).

In order to test for interspecific differenees in walking or climbing speeds, we employed two separate single factor ANOVAs. Because we used the same individuals for both walking and climbing, we used a Bonferroni correction to calculate an adjusted alpha level (α = 0.025). For significant differences detected by the ANOVA, we applied a post-hoc

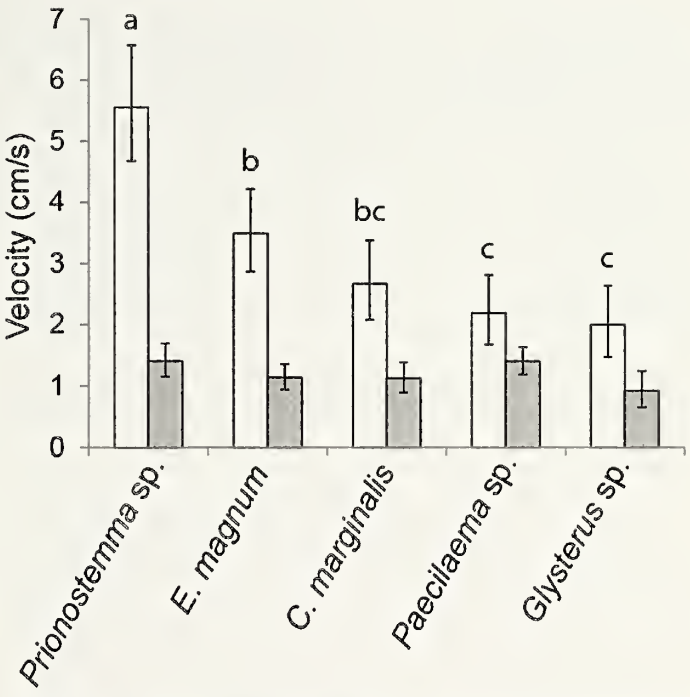

Figure 1.—Mean walking (white bars) and climbing (gray bars) speeds with 95% CI for five species of harvestmen. Significant differences among species are indicated with different letters.

Tukey Honest Significant Differences method to determine which species differed. Additionally, to test the hypothesis that walking and climbing rates are correlated for each of the five species we utilized pairwise Mann-Whitney U tests (α = 0.05). Means and 95% confidence intervals for walking and climbing speeds (cm/s) were calculated from log-transformed data and back-transformed (Fig. 1).

In the species of harvestmen examined in our study, legs III and IV are important in terrestrial locomotion, similar in relative length, and both are significantly longer than leg I. We used the mean length of leg IV to test for correlations between walking speed and leg morphology. Using digital calipers, we measured the total length of leg IV (mm) for all specimens tested. Despite multiple attempts to transform the data, the residuals for the leg length data were not normally distributed, and variances were not homogeneous. Therefore, we analyzed these morphometric data using Kruskal-Wallis rank sum tests followed by Mann-Whitney U tests to test the null hypothesis that leg IV length does not differ between species. We report means and standard deviation as a measure of central tendency since mean values did not differ greatly from the medians, and because other data are expressed as means.

We calculated species means from log transformed data for leg length, walking speed and climbing speed, back-transformed the means and 95% CI, and employed regression analyses to determine if mean leg length affected mean walking and climbing speeds. We used means because leg length data were not collected at the time of walking and climbing trials, and thus leg length measurements were not paired with time trials for each individual. We tested the null hypotheses that there is no relationship between leg length and walking or climbing speed (the slopes of the regression lines equaled zero). This provided a preliminary basis for assessing the effects of leg

Table 1.—Ecological and morphological comparisons of Costa Rican harvestmen examined in this study. For climbing status, species are classified as arboreal (A), semi-arboreal (S), or rarely arboreal (R). General habitat preferences are provided for day (D) and night (N) and are based upon the field data presented by Wade et al. (2011) and Proud et al. (2012). For morphological characteristics, we calculated the mean ± SD for scutal length and for the length of leg IV. Samples sizes are $n = 24$ for *Glysterus* sp., and $n = 30$ for each of the other four species.

Species	Climb status	Habitat preferences	Scutal length (mm)	Length of leg IV (mm)
C. marginalis	A	Terrestrial (D, N) Arboreal (D, N)	4.2 ± 0.4	38.0 ± 6.6
E. magnum	S	Terrestrial (D, N) Arboreal (N)	6.8 ± 0.5	57.4 ± 7.6
Paecilaema sp.	S	Terrestrial (D, N) Arboreal (N)	4.9 ± 0.3	30.4 ± 2.4
Glysterus sp.	R	Terrestrial (D, N)	5.0 ± 0.4	14.7 ± 1.8
Prionostemma sp.	A	Arboreal (D) Terrestrial (N)	4.6 ± 0.8	66.7 ± 6.1

length on walking and climbing speeds for these species. All statistical analyses were implemented in R (R Development Core Team 2010).

RESULTS

Walking speed differed significantly among species ($F = 13.69$, $df = 4$, $P < 0.001$); however, no significant differences were detected for climbing speeds ($F = 1.97$, $df = 4$, $P = 0.105$). The pairwise post-hoc comparisons indicated that the mean walking speed of *Prionostemma* sp. was significantly faster than all other species (Fig. 1). Additionally, *E. magnum* walked significantly faster than *Paecilaema* sp. and *Glysterus* sp., but no differences were detected in walking speed between *Paecilaema* sp., *C. marginalis* and *Glysterus* sp. (Fig. 1). Results of the Mann-Whitney U tests revealed that all species walked significantly faster than they climbed (Fig. 1; all $df = 1$, *Prionostemma*: $U = 40.4$, $P < 0.001$; *E. magnum*: $U = 27.5$, $P < 0.001$; *Paecilaema* : $U = 19.5$, $P < 0.001$; *C. marginalis*: $U = 5.39$, $P = 0.02$; *Glysterus*: $U = 7.01$, $P = 0.008$).

Relative leg length (Table 1) differed significantly among species (Kruskal-Wallis $\chi^2 = 133.7$, $df = 4$, $P < 0.001$). *Prionostemma* sp. had the longest leg length followed by *E. magnum*, *C. marginalis* and *Paecilaema* sp., respectively. *Glysterus* sp. had the shortest leg length.

We rejected the null hypothesis that the slope of the line $\beta_1 = 0$ for the regression of mean walking speed against mean leg length: $r^2 = 0.771$, $df = 3$, $P = 0.032$, regression equation $y = 0.569 + 0.635x$, where y = walking speed (cm/s) and x = length of leg IV (cm) (Fig. 2A). Mean leg length had no significant effect on mean climbing speed, and we were not able to reject the null hypothesis that $\beta_1 = 0$: $r^2 = 0.10$, $df = 3$, $P = 0.32$, regression equation $y = 0.965 + 0.056x$, where y = climbing speed (cm/s) and x = length of leg IV (cm) (Fig. 2B).

DISCUSSION

There are relatively few published walking or climbing speeds for harvestmen (Houghton et al. 2011). Schmitz (2005) examined the impact of movement upon metabolic rate using running velocities up to 96 cm/min for two species. Guffey (1999) and Houghton et al. (2011) assessed the impact of leg autotomy upon walking speeds for sclerosomatid species and reported velocities up to 9.3 cm/s for individuals with all eight

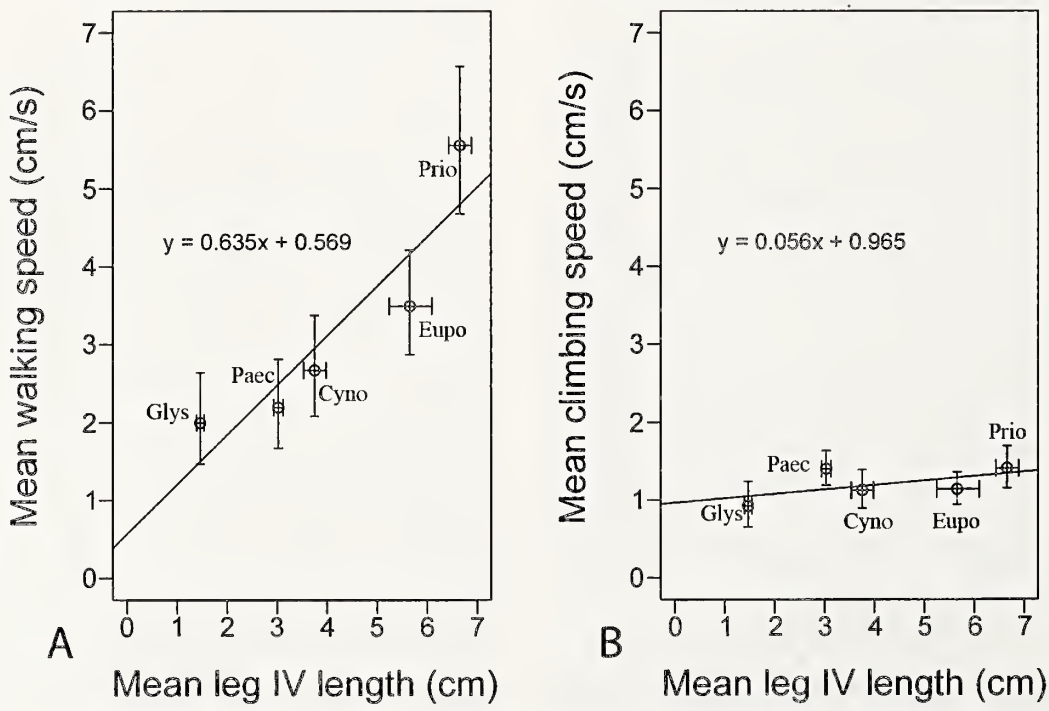

Figure 2.—Regression of mean leg IV length on speed with 95% CI for five species of harvestmen. A) Mean walking speed as a function of mean leg IV length. B) Mean climbing speed as a function of mean leg length. Abbreviations: Glys = *Glysterus* sp.; Paec = *Paecilaema* sp.; Cyno = *Cynorta marginalis*; Eupo = *Eupoecilaema magnum*; Prio = *Prionostemma* sp.

legs intact on a flat, horizontal surface. Our data indicate that species with relatively longer legs walk significantly faster than species with shorter legs, but that climbing speed is not affected by leg length. The mean lengths for leg IV of *Prionostemma* sp. and *E. magnum* exceed 55 mm, whereas those for *C. marginalis*, *Paecilaema* sp. and *Glysterus* sp. are much shorter.

Our results also indicate that harvestmen walk significantly faster than they climb, regardless of the habitat in which they are most frequently encountered. In addition, climbing speed did not significantly vary between species, regardless of relative leg length. Curtis and Machado (2007) cautiously reported a functional relationship between leg morphology and the use of arboreal habitats for sclerosomatid harvestmen. In our study, the arboreal *C. marginalis* (short legs) and *Prionostemma* (long legs) sp. climbed at speeds that were not significantly different from each other or from that of the leaf litter residing *Glysterus* (short legs).

In our study, the most arboreal species use the surfaces of trees in different ways. During the day, adult *C. marginalis* occupying perches on trunks or buttresses are inactive (Wade et al. 2011). However, after dusk they become active and climb, interact with conspecifics and forage (Wade et al. 2011). In contrast, adult and nymphs of *Prionostemma* sp. assemble in loose aggregations on arboreal perches during the day and descend to the leaf litter after dusk, presumably to forage (Wade et al. 2011).

Interspecific variation in walking speeds may reflect different strategies for life in the leaf litter microhabitat. Individuals of *Prionostemma* sp., the most vagile species in our sample, are capable of moving considerable distances (up to 0.2 km/night) in forested habitats (Donaldson & Grether 2007, Grether & Donaldson 2007). These harvestmen may rely in part upon fast walking speeds to elude potential invertebrate and vertebrate predators (Gnaspini & Hara 2007). Shorter-legged, less vagile species such as *C. marginalis*, *Paecilaema* sp., and *Glysterus* sp. may use slow movement to avoid detection by visually oriented predators (Gnaspini & Hara 2007). More detailed natural history studies of each of these species are required to assess these hypotheses.

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Epigeal spider responses to fertilization and plant litter: testing biodiversity theory at the ground level

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Abstract. Recent studies of nutrient additions to terrestrial ecosystems have focused on the "aerial" portion of the food web associated with living plants. These studies showed nutrient loading increased arthropod abundance and biomass, but decreased diversity. However, none of these studies explicitly examined nutrient loading effects on epigeal arthropods. To test nutrient loading effects on epigeal spiders and on individual species within a temperate-latitude grassland community, we used pitfall traps to sample spiders for four years within 24 large (314 m^2) plots in which we manipulated nutrients (NPK fertilizer) and plant litter (litter removed or left in place). We measured the diversity, abundance, biomass, and community structure responses of the spider community, and of wolf spiders (Lycosidae) and linyphiid spiders (Linyphiidae), as well as the abundance and biomass responses of the six most common species. We hypothesized increased nutrient loading would increase epigeal spider abundance and biomass but decrease diversity. Contrary to predictions, spider species richness, diversity, and biomass were not significantly affected by fertilization, while fertilization resulted in significantly increased abundance. Also contrary to predictions, plant litter did not affect any of these variables. Linyphiid spiders had the strongest responses to fertilization, with significantly increased abundance and biomass, and, contrary to predictions, increased species richness in fertilized plots. Wolf spiders responded more closely to predictions. Our results indicate that the epigeal spider community does not respond as would be predicted by biodiversity-productivity theory. This underscores the need to integrate the largely detritus-based epigeal community into current biodiversity-productivity theory.

Keywords: Araneae, Linyphiidae, Lycosidae, enrichment, disturbance, nitrogen

Human activity has resulted in a significant increase in the global nitrogen (N) pool through fertilization and increased atmospheric N deposition (Vitousek et al. 1997; Fenn et al. 2003; Galloway et al. 2003). Typical plant community responses include decreased plant species richness, increased standing crop biomass, and the limitation of community composition to a few dominant species (Hector et al. 1999; Tilman et al. 2002; Suding et al. 2005; Patrick et al. 2008a). This research has provided substantive support for biodiversity-productivity theory, which predicts declines in local and regional richness as one moves from mesotrophic to eutrophic systems (Grime 1973; McCann 2000; Worm & Duffy 2003; Suding et al. 2005; Chalcraft et al. 2008). Similarly with terrestrial arthropods, increased nutrient loading has been linked with decreased species richness and increased abundance, particularly among those species most closely linked to the living-plant portion of the food web (Knops et al. 1999; Haddad et al. 2000, 2001; but see Patrick et al. 2008b). This "eutrophication effect" (Fenn et al. 2003) can result in significant biodiversity loss and potential decline in important ecosystem functions, such as ecosystem stability (McCann 2000; Larsen et al. 2005).

Previous studies of nutrient loading have focused on the portions of the food web closely tied to living plant material; e.g., the "aerial" arthropod community associated with the upper portions (e.g., stems) of plants (Knops et al. 1999; Haddad et al. 2000, 2001). Although there is evidence to support the eutrophication effect on aerial arthropod diversity, less is known regarding how nutrient loading affects the epigeal (ground-level) arthropod community. A differential response by the epigeal arthropod community may result from it being more closely tied to the detritus-based portion of the food web (Halaj & Wise 2002). Despite the important role it may play, the epigeal arthropod community remains an understudied food web component (Wardle 2002; Hättenschwiler et al. 2005; Cross et al. 2006).

Nutrient loading also increases plant litter production (Long et al. 2003; Patrick et al. 2008a), which can increase the basal food resource for the detrital community and increase detritivore and epigeal predator abundances (Halaj et al. 2000; Halaj & Wise 2002; Moore et al. 2004). Furthermore, plant litter increases habitat complexity, which can also increase arthropod abundance and diversity (Lawton 1983; Strong et al. 1984; Rypstra et al. 1999). Although more plant litter production could increase detritivore and epigeal predator abundance and biomass (Halaj et al. 2000), a reduction in litter diversity could result in decreased diversity of detritivores and epigeal predators (Hättenschwiler & Gasser 2005; Wardle 2006), mirroring the aerial community response to nutrient loading.

Spiders, in particular, are abundant generalist predators (Wise 1993) that can significantly impact terrestrial food webs (Wise et al. 1999), and epigeal spiders (e.g., Lycosidae and Linyphiidae) are closely linked to the detritivore community (Wise et al. 1999; Chen & Wise 1999; Wise 2006). The abundance of epigeal spiders is limited ultimately by the abundance of their mainly detritivorous prey via bottom-up forces through the detritus-based portion of the food web (Chen & Wise 1999; Wise et al. 1999; Wise 2004, 2006). Thus, increasing plant detritus can increase spider abundance by increasing the quantity of food available to their detritivorous prey (Chen & Wise 1999; Wise et al. 1999; Wise 2004). Increased detritus also enhances habitat structure for hiding and web building (Uetz 1979, 1991; Rypstra et al. 1999), which

can also moderately increase the local richness of the spider community (Rypstra et al. 1999), also differentially affecting individual spider species.

Even though spider abundance may increase, spider diversity may not increase proportionally because the redueed diversity of plant detritus can limit predator diversity in the detrital food web (Hättensehwiler & Gasser 2005; Wardle 2006). Thus, it is reasonable to expect that predators dependent upon the detritivore food web may have the same response to fertilization as predators more closely associated with the aerial food web. Even though more plant litter is produced, increasing the resource base of the detritivore food web, lower litter diversity likely begets lower detritivore and detritivore-predator diversity (Hättenschwiler & Gasser 2005; Wardle 2006). Interestingly, no epigeal spider studies (focused strictly on cursorial spiders; e.g., wolf spiders) have looked at the spider diversity response to basal resource manipulation. Moreover, no studies have examined responses of the predominantly epigeal spider family Linyphiidae (wandering sheet/tangle-web builders) that may patrol multiple webs at ground level (Uetz et al. 1999).

Here we report the results of a four-year study that investigated the response of the epigeal spider community to experimental manipulations of NPK fertilization and plant litter availability in a temperate-latitude grassland. We measured the diversity, abundance, biomass, and eommunity structure responses of the entire epigeal spider community, the spider families Lycosidae and Linyphiidae, and the dominant individual spider species. Our goal was to integrate the detrital food web into biodiversity-productivity theory through insight gained from the responses of predators that rely largely on the detritivore food web. Based on previous studies that sampled the aerial arthropod eommunity responses to nutrient loading (e.g., Knops et al. 1999; Haddad et al. 2000, 2001), we tested two hypotheses: (1) fertilization will cause spider biomass and abundance to increase and spider species richness to decrease, and (2) the presenee of plant litter will moderately increase spider species richness, though this effect will be dampened in fertilized plots.

METHODS

Study site and experimental design.—The study was done at the 163.5 ha Bath Nature Preserve (BNP: 41°10′36.2″N, 81°38′58.7″W), Bath Township, Summit County, Ohio, USA, in a 16 ha section of grassland. Until the early 1980s, the study site was a hay meadow, harvested one or many times per year. Since then, the area has been mown annually in late August to early September, and the mown vegetation has been left on the field. The dominant vegetation is an herbaceous, graminoid community largely dominated by cool-season C_3 grasses, e.g., *Bromus inermis* Leyss., *Lolium arundinaceum* (Schreb.) Darbysh, *Phleum pratense* L., and *Anthoxanthum odoratum* L. The site is moderately productive relative to other grasslands within the upper Midwest and across the USA (Patrick et al. 2008a). The dominant soil type is Ellsworth silt loam (ElB), which consists of moderately well drained, moderately deep to deep soils formed in silty clay loam or clay loam glacial till of the Wisconsin Age (Ritchie & Steiger 1974).

During August 2001, twenty-four 20-m diameter circular plots (314 m^2) were established. These experimental plots were separated by at least 20 m and were at least 30 m away from any other habitat. Treatments were applied in a 2 × 2 factorial design of fertilizer (+F = fertilizer added, −F = no fertilizer) and plant litter (−L = litter removed, +L = litter left in situ after yearly mowing) with control plots characterized as no fertilization and plant litter left in situ (+L/−F), resulting in six replicates per treatment. Hereafter, all references to "litter" refer to the previous year's mown vegetation and any vegetation senesced and found within the sampling quadrat after standing crop removal. In April 2002 and continuing each April through 2005, Scotts brand Osmocote 8–9 month Slow Release Fertilizer 19-6-12 (NPK; Scotts, Marysville, Ohio USA) was applied at 20 gNm^{-2} in fertilized plots, well above the Köchy & Wilson (2005) 15 g Nm^{-2} yr^{-1} threshold necessary to induce a eutrophication effect in grasslands and other habitats. We could not exclude ambient wet/dry atmospheric N deposition, though deposition rates from 1990 to 2005 were relatively low at ~1.01 g N m^{-2} yr^{-1} at a nearby monitoring site in Lykens (162 km west of our study site), Ohio, USA, and ~0.93 g N m^{-2} yr^{-1} at another nearby monitoring site in Mercer Co. (G.K. Goddard site, 96 km east of our study site), Pennsylvania, USA (US EPA 2005). Within two days of annual mowing of the whole site by the local township with a large tractor and brush hog mower (autumn 2001–2004), litter was removed from litter-removal treatments using a small 23 hp lawn tractor with a pull-behind 8 hp Agri-Fab Mow-N-Vac trailer attachment (Agri-Fab, Sullivan, Illinois, USA).

Spider community sampling.—Spiders were collected using four pitfall traps in each of the 24 experimental plots (n = 96 total pitfall traps). Within each plot, a single trap was placed 5 m from the center of the plot at each of four magnetic compass directions (northeast, northwest, southeast, and southwest). Each trap consisted of a 10 cm diameter, 18 cm tall PVC sleeve into which a 710-mL plastic cup was inserted and filled to ~4 cm with a 50/50 water/propylene glycol mixture. To deter trap raiders (e.g., microtine mammals), prevent captured spiders from climbing out of the trap, and prevent precipitation from directly flooding the trap, an 8-cm powder funnel with a base enlarged to ~ 3 cm was inserted and a 15 cm × 15 cm board was placed over each trap, leaving ~ 3 cm clearance. Starting mid to late May (mid-July during 2004) and continuing through mid to late August, traps were alternately left open for two weeks and closed for two weeks. This resulted in three sampling periods each year during 2002, 2003, and 2005. During 2004, only the second and third sampling periods were collected. When each two-week sampling period ended, the plastic cups were removed, the contents collected and preserved in 70% ethyl alcohol, and the PVC sleeve was tightly capped. Although pitfall traps do not capture all spiders in the community, they are an effective sampling technique for determining the relative abundanee and species richness of epigeal spiders (Greenslade 1964; Phillips & Cobb 2005). Spiders captured in each trap were identified to speeies for all mature specimens (taxonomic names follow Platniek 2012), and to family for all immature specimens, and exact numbers of species/families within in each trap were recorded and dried at 70°C for 72 h to determine speeies-specific biomasses to the nearest 0.0001 g. Lacking sufficient numbers captured within a trap, some

extremely small species did not register a biomass, and their biomass was recorded as "0.000001 g" to differentiate them from true zeroes in analyses.

Statistical analyses.—We tested the effects of fertilization, plant litter, and the interaction of fertilization and litter on the abundance, biomass, species richness (SR), and effective Shannon's diversity ($e^{H'}$, where H' is the Shannon diversity index) of (1) all mature spiders (Araneae), (2) lycosid and linyphiid spiders and (3) abundance and biomass of the six most abundant spider species. We used $e^{H'}$ to correct for differences in species richness that might have resulted from differential spider abundances (Ricklefs & Miller 2000; Haddad et al. 2000). To calculate the average SR within a plot, we summed the total number of spider species caught in each trap, then averaged this SR for each of the four traps within a plot within a sampling period (including zeroes for traps where no spiders were captured), then averaged these SRs for each plot across sampling periods in a year, yielding $n = 24$ samples within each year. The same method was used to calculate the average abundance, biomass and $e^{H'}$ within a plot within a year, also yielding $n = 24$ samples within each year. Correlations and regressions of these spider responses with plant species richness (plant SR) and standing crop biomass utilized data from Patrick et al. (2008a).

To analyze trends per year and per treatment in abundance, biomass, SR, and $e^{H'}$ we used SAS software version 8.01 (SAS Institute, Inc. 1999) to calculate a Generalized Linear Mixed-effect Model (GLMM) in PROC MIXED with Type III effects based upon the covariance structure of compound symmetry, and Gaussian distribution of errors. The various models used the different response variables (biomass, SR, $e^{H'}$, abundance), and for the predictor variables used fertilized vs. unfertilized, litter removed vs. litter left in situ, year, and their factorial interactions, with year as the repeated predictor. When year was detected as a significant effect for a response variable, we tested for treatment effects within a year and again used SAS to calculate a GLMM in PROC MIXED with Type III effects based upon the covariance structure of compound symmetry, Gaussian distribution of error, with fertilization, litter, and the factorial interaction of fertilization and litter as predictor variables.

To assess treatment effects on aggregate biotic and abiotic components in our system, we applied nonmetric multidimensional scaling (NMS: Kruskal 1964) using PC-ORD (McCune & Mefford 2006). For 2005, variables used for each of the 24 plots were average spider species richness per plot and four variables used in a previously published analysis (Patrick et al. 2008a): average plant litter biomass, average PAR per plot, average percent soil moisture per plot, and average percent soil organic content per plot, resulting in a matrix with five columns and 24 rows (plots). The same analysis was run a second time with the same variable, except Linyphiidae species richness replaced spider species richness. Because (1) NMS is scale sensitive, (2) these variables are on radically different measurement scales, and (3) variables have an enormous range of values between variables, data were transformed to proportions relative to the highest value for each variable (i.e., each value in a column was divided by the largest value in that column, creating a unitless range from 0–1 for each column). The NMS analysis was run with Sørensen distance, time as the random seed for the starting configuration, 9999 runs stepping down from 5 to 1 dimensions with the real data, 999 Monte Carlo runs to assess the probability of a similar final stress obtained by chance, and a 0.005 stability criterion. Additionally for 2005 and to support NMS analyses with stable results, we used PC-ORD (McCune & Mefford 2006) to run the multi-response permutation procedure (MRPP: Mielke 1984) to test for the hypothesis of no difference among treatments. The MRPP used Sørensen distance with the four treatments as the a priori groupings, resulting in a matrix with five columns (biotic and abiotic variables) and 24 rows (plots) and was calculated with all four treatments together, and for pairwise comparisons between treatments to test for the strength of difference between individual treatments.

RESULTS

General trends.—A total of 13,174 spiders from 14 families was captured during 14,784 trap nights. Of this total, 2515 spiders were immature and from 11 families, while the remaining 10,659 spiders were mature and from 94 species and 12 families (Table 1). Lycosidae was the most commonly captured spider family, with 6577 mature specimens (61.7% of all mature spiders captured) from 20 species, while Linyphiidae was the second most commonly captured spider family with 3200 mature specimens (30.0% of all mature spiders captured) from 34 species. Together these two families represented 9777 (91.7% of all mature spiders captured) specimens from 54 species (57.4% of all species captured). Spider diversity, corrected for abundance with $e^{H'}$, was significantly affected by fertilization and by year, but not by litter (Table 2). The factorial interactions between fertilization and year, fertilization and litter, litter and year, and the fully factorial interaction of fertilization by litter by year were not significant (Table 2). Because of the significance of year (Fig. 1), we tested for treatment effects on spider diversity within each year and by 2005 (Table 3) neither fertilization, nor litter nor their interaction was significant.

Fertilization significantly affected Araneae (all spiders) abundance but not Araneae SR or Araneae biomass (Table 2, Fig. 2A– C). Moreover, fertilization effects were significant for Linyphiidae SR, abundance and biomass (Table 2, Fig. 2D–F), as well as for Lycosidae SR and abundance (Table 2, Fig. 2G–H), but not for Lycosidae biomass (Table 2, Fig. 2I). All response variables were significantly affected by year (Table 2), and Araneae SR and abundance, and Linyphiidae SR, abundance and biomass had significant fertilization and year interactions.

Neither Araneae SR nor Lycosidae SR were significantly correlated with abundance ($r = 0.335$ and $r = -0.190$, respectively), but Linyphiidae SR was well correlated with abundance ($r = 0.857$; Fig. 3A–C). As with abundance, biomass was only correlated in the Linyphiidae SR ($r = 0.629$; Fig. 3D–F). Although Araneae SR was not significantly correlated with plant SR ($r = 0.276$; Fig 4A), Linyphiidae SR was negatively correlated with plant SR ($r = -0.400$), and Lycosidae SR was positively correlated with plant SR ($r = 0.639$; Fig. 4B–C). Araneae SR was also not correlated with standing crop biomass ($r = 0$; Fig. 4D), while Linyphiidae SR was positively correlated and Lycosidae SR was negatively correlated with standing crop biomass ($r = 0.629$ and $r = -0.425$, respectively; Fig. 4E–F).

Table 1.—Total numbers of each family and species of spider captured during the four year manipulative experiment. "+L/−F" represents the control treatment of unfertilized plots with litter left in situ, "−L/−F" represents the unfertilized with litter removed treatment, "+L/+F" represents the fertilized with litter left in situ treatment, "−L/+F" represents the fertilized with litter-removed treatment, and "Total" represents the total number caught. Immature spiders were classified as "unidentified," and family names are in bold.

Family/Species	+L/−F	−L/−F	+L/+F	−L/+F	Total
Agelenidae					
unidentified	1	0	0	0	1
Araneidae					
unidentified	1	2	0	0	3
Clubionidae	**15**	**15**	**4**	**3**	**37**
Clubiona abboti L. Koch 1866	0	0	1	0	1
Clubiona kastoni Gertsch 1941	6	9	1	1	17
unidentified	9	6	2	2	19
Corinnidae	**90**	**71**	**29**	**26**	**216**
Castianeira gertschi Kaston 1945	3	5	0	0	8
Castianeira longipalpa (Hentz 1847)	1	0	0	1	2
Castianeira variata Gertsch 1942	1	0	1	0	2
Meriola decepta Banks 1895	10	13	3	6	32
Phrurotimpus borealis (Emerton 1911)	0	0	1	0	1
Scotinella britcheri (Petrunkevitch 1910)	0	0	0	2	2
Scotinella fratrella (Gertsch 1935)	71	42	24	17	154
Scotinella madisonia Levi 1951	3	11	0	0	14
unidentified	1	0	0	0	1
Dictynidae					
Cicurina arcuata Keyserling 1887	0	0	2	1	3
Gnaphosidae	**139**	**104**	**77**	**77**	**397**
Drassyllus creolus Chamberlin & Gertsch 1940	12	12	3	2	29
Drassyllus depressus (Emerton 1890)	63	57	20	30	170
Gnaphosa parvula Banks 1896	43	19	44	37	143
Litopyllus temporarius Chamberlin 1922	0	0	2	1	3
unidentified	21	16	8	7	52
Hahniidae	**5**	**17**	**4**	**2**	**28**
Neoantistea agilis (Keyserling 1887)	0	1	2	0	3
Neoantistea magna (Keyserling 1887)	2	4	2	1	9
Neoantistea riparia (Keyserling 1887)	1	8	0	0	9
unidentified	2	4	0	1	7
Linyphiidae	**717**	**532**	**1131**	**1058**	**3438**
Agyneta sp. 1	0	1	0	0	1
Agyneta sp. 2	1	0	0	0	1
Agyneta sp. 3	0	0	0	1	1
Allomengea dentisetis (Grube 1861)	0	0	1	1	2
Bathyphantes pallidus (Banks 1892)	127	64	336	232	759
Centromerus cornupalpis (O. P.-Cambridge 1875)	4	0	11	4	19
Ceraticelus similis (Banks 1892)	0	1	1	0	2
Ceratinopsis laticeps Emerton 1882	0	0	0	1	1
Collinsia plumosa (Emerton 1882)	31	20	150	192	393
Diplostyla concolor (Wider 1834)	3	8	117	42	170
Eridantes erigonoides (Emerton 1882)	242	126	195	225	788
Erigone autumnalis Emerton 1882	56	51	33	45	185
Erigone dentigera O. P.-Cambridge 1874	0	1	1	2	4
Grammonota gentilis Banks 1898	0	0	0	1	1
Grammonota inornata Emerton 1882	20	51	13	17	101
Islandiana flaveola (Banks 1892)	11	12	8	7	38
Maso sundevalli (Westring 1851)	1	0	0	0	1
Meioneta fabra (Keyserling 1886)	10	13	6	9	38
Meioneta micaria (Emerton 1882)	5	4	2	0	11
Meioneta minuta (Banks 1892)	85	52	61	72	270
Mermessus entomologicus (Emerton 1911)	0	1	0	0	1
Mermessus jona (Bishop & Crosby 1938)	9	9	8	2	28
Mermessus tridentatus (Emerton 1882)	3	2	1	3	9

Table 1.—Continued.

Family/Species	+L/−F	−L/−F	+L/+F	−L/+F	Total
Mermessus trilobatus (Emerton 1882)	38	26	55	56	175
Neriene clathrata (Sundevall 1830)	23	8	2	6	39
Oedothorax trilobatus (Banks 1896)	0	0	2	5	7
Tennesseellum formica (Emerton 1882)	0	1	0	0	1
Tenuiphantes tenuis (Blackwall 1852)	0	0	0	5	5
Walckenaeria directa (O. P.-Cambridge 1874)	3	0	6	3	12
Walckenaeria palustris Millidge 1983	0	0	1	0	1
Walckenaeria sp. 1	1	0	0	0	1
Walckenaeria sp. 2	0	0	1	0	1
Walckenaeria spiralis (Emerton 1882)	12	28	30	63	133
Walckenaeria tibialis (Emerton 1882)	1	0	0	0	1
unidentified	31	53	90	64	238
Liocranidae					
Agroeca pratensis Emerton 1890	1	0	0	0	1
Lycosidae	**1913**	**2021**	**2422**	**2276**	**8632**
Allocosa funerea (Hentz 1844)	19	15	2	14	50
Hogna helluo (Walckenaer 1837)	0	6	1	1	8
Pardosa milvina (Hentz 1844)	0	3	0	0	3
Pardosa modica (Blackwall 1846)	2	1	6	3	12
Pardosa moesta Banks 1892	344	233	1444	1177	3198
Pardosa saxatilis (Hentz 1844)	140	132	16	73	361
Pirata aspirans Chamberlin 1904	0	1	0	0	1
Pirata sedentarius Montgomery 1904	0	1	1	10	12
Piratula canadensis (Dondale & Redner 1981)	0	1	0	0	1
Piratula gigantea (Gertsch 1934)	0	0	0	1	1
Piratula insularis (Emerton 1885)	7	27	10	22	66
Piratula minuta (Emerton 1885)	365	638	538	395	1936
Rabidosa punctulata (Hentz1844)	2	3	0	0	5
Rabidosa rabida (Walckenaer 1837)	4	3	0	0	7
Schizocosa avida (Walckenaer 1837)	33	94	4	9	140
Schizocosa bilineata (Emerton 1885)	80	61	25	34	200
Schizocosa crassipalpata Roewer 1951	113	108	43	71	335
Trochosa ruricola (De Geer 1778)	24	16	36	37	113
Trochosa terricola Thorell 1856	18	29	42	36	125
Varacosa avara (Keyserling 1877)	2	1	0	0	3
unidentified	760	648	254	393	2055
Philodromidae					
Ebo latithorax Keyserling 1884	0	1	0	0	1
Salticidae	**63**	**75**	**16**	**21**	**175**
Ghelna barrowsi (Kaston 1873)	1	2	0	1	4
Ghelna canadensis (Banks 1897)	4	3	0	2	9
Ghelna castanea (Hentz 1846)	2	0	0	0	2
Marpissa lineata (C.L. Koch 1846)	7	3	1	2	13
Myrmarachne formicaria (De Geer 1778)	0	1	0	1	2
Neon avalonus Gertsch & Ivie 1955	1	0	0	0	1
Neon nelli Peckham & Peckham 1888	7	12	1	2	22
Neon plutonus Gertsch & Ivie 1955	25	35	7	7	74
Sarinda hentzi (Banks 1913)	0	1	0	0	1
Talavera minuta (Banks 1895)	11	7	2	3	23
unidentified	5	11	5	3	24
Tetragnathidae	**51**	**46**	**16**	**22**	**135**
Glenognatha foxi (McCook 1894)	9	10	3	8	30
Pachygnatha autumnalis Marx 1884	17	14	9	6	46
Pachygnatha clerki Sundevall 1823	0	0	0	1	1
Pachygnatha xanthostoma C.L. Koch 1845	0	0	1	0	1
Tetragnatha laboriosa Hentz 1850	1	0	0	0	1
unidentified	24	22	3	7	56

Table 1.—Continued.

Family/Species	+L/−F	−L/−F	+L/+F	−L/+F	Total
Thomisidae	**19**	**54**	**14**	**20**	**107**
Xysticus bicuspis Keyserling 1887	1	0	0	0	1
Xysticus canadensis Gertsch 1934	1	1	0	0	2
Xysticus ferox (Hentz 1847)	4	25	7	9	45
Xysticus fraternus Banks 1895	0	1	0	0	1
Xysticus luctans (C.L. Koch 1845)	0	0	1	0	1
unidentified	13	27	6	11	57

Spider species-level analyses.—The most abundant spider was *Pardosa moesta* Banks 1892, with 3198 mature specimens captured (30.0% of all mature spiders, 48.6% of mature lycosids). *Pardosa moesta* was easily the most captured spider in fertilized plots although being virtually absent from unfertilized plots (Fig. 5A), and had a strong response to fertilization and year, resulting in a significant fertilization by year interaction (Table 3). A smaller lycosid, *Piratula minuta* (Emerton 1885), was the second most abundant spider with 1936 mature specimens captured (18.2% of all mature spiders, 29.4% of mature lycosids) but did not seem to specifically and consistently respond to a particular treatment (Fig. 5B), though year and the fertilization by litter interaction were significant (Table 3). Together, these two species accounted for nearly half (48.2%) of all mature spiders captured, and over three-quarters (78%) of all mature lycosid spiders.

The third most abundant spider was the linyphiid *Eridantes erigonoides* (Emerton 1882), with 788 mature specimens captured (7.4% of all mature spiders, 24.6% of mature linyphiid spiders). Similar to *Pi. minuta*, *E. erigonoides* did not consistently respond to any particular treatment (Fig. 5C), but did have a significant response to year in the repeated-measures analysis (Table 3). However, the fourth most abundant spider, the linyphiid *Bathyphantes pallidus* (Banks 1892) with 759 mature specimens captured (7.1% of all mature spiders, 23.7% of linyphiid spiders), strongly responded to fertilization, year, and the interaction between fertilization and year, and responded marginally significantly to litter (Fig. 5D; Table 3). Virtually absent from the study site during the first two years of the study, *B. pallidus* became a fairly common spider in fertilized plots during the final two years of the study, with a weak affiliation to plots where litter was left in situ (Fig. 5D). Also virtually absent from the site during the first two years of the study, the fifth most abundant spider, the linyphiid *Collinsia plumosa* (Emerton 1882) with 393 specimens captured (3.7% of all mature spiders, 12.3% of mature linyphiid spiders), also strongly responded to fertilization and year, which resulted in a significant interaction between fertilization and year (Fig. 5E; Table 3).

One of the most abundant of the larger spiders, the lycosid *Schizocosa avida* (Walckenaer 1837) with 140 mature specimens captured (1.3% of all mature spiders, 2.1% of mature lycosid spiders), was virtually absent from fertilized plots (Fig. 5F). All variables were significant at the $\alpha < 0.05$ level (Table 3). The species was most commonly captured in litter-removed treatments, particularly in unfertilized plots with litter removed (−L/−F).

Table 2.—Results of the GLMM for each response variable. Given are the *F* values with degrees of freedom and the resulting *P* values, where bolded values indicate significance at $\alpha < 0.0007$ (after applying Bonferroni correction). The predictor variables are fertilization (F), litter (L), year (Y), and (*) their fully factorial interactions. Species richness is indicated by "SR."

Response Variable	F	L	Y	F*L	F*Y	L*Y	F*L*Y
e^H	$F_{1, 80} = 6.6$ $P = 0.0124$	$F_{1, 80} = 0.8$ $P = 0.39$	$F_{3, 80} = 80.0$ **$P < 0.0001$**	$F_{1, 80} = 2.2$ $P = 0.14$	$F_{3, 80} = 1.4$ $P = 0.25$	$F_{3, 80} = 2.3$ $P = 0.08$	$F_{3, 80} = 0.2$ $P = 0.89$
Araneae SR	$F_{1, 80} = 1.0$ $P = 0.33$	$F_{1, 80} = 0.5$ $P = 0.48$	$F_{3, 80} = 87.1$ **$P < 0.0001$**	$F_{1, 80} = 1.5$ $P = 0.23$	$F_{3, 80} = 3.4$ $P = 0.0231$	$F_{3, 80} = 1.6$ $P = 0.19$	$F_{3, 80} = 0.3$ $P = 0.84$
Araneae abundance	$F_{1, 80} = 39.64$ **$P < 0.0001$**	$F_{1, 80} = 1.0$ $P = 0.32$	$F_{3, 80} = 54.9$ **$P < 0.0001$**	$F_{1, 80} = 0.7$ $P = 0.42$	$F_{3, 80} = 5.2$ $P = 0.0024$	$F_{3, 80} = 0.1$ $P = 0.98$	$F_{3, 80} = 0.8$ $P = 0.48$
Araneae biomass	$F_{1, 80} = 0.0$ $P = 0.84$	$F_{1, 80} = 1.6$ $P = 0.21$	$F_{3, 80} = 38.1$ **$P < 0.0001$**	$F_{1, 80} = 2.3$ $P = 0.14$	$F_{3, 80} = 1.5$ $P = 0.23$	$F_{3, 80} = 1.6$ $P = 0.19$	$F_{3, 80} = 2.0$ $P = 0.12$
Linyphiidae SR	$F_{1, 80} = 28.1$ **$P < 0.0001$**	$F_{1, 80} = 0.2$ $P = 0.66$	$F_{3, 80} = 53.1$ **$P < 0.0001$**	$F_{1, 80} = 2.8$ $P = 0.10$	$F_{3, 80} = 9.7$ **$P < 0.0001$**	$F_{3, 80} = 1.9$ $P = 0.14$	$F_{3, 80} = 0.4$ $P = 0.76$
Linyphiidae abundance	$F_{1, 80} = 20.9$ **$P < 0.0001$**	$F_{1, 80} = 1.5$ $P = 0.22$	$F_{3, 80} = 17.3$ **$P < 0.0001$**	$F_{1, 80} = 0.7$ $P = 0.40$	$F_{3, 80} = 6.6$ **$P = 0.0005$**	$F_{3, 80} = 0.1$ $P = 0.97$	$F_{3, 80} = 0.2$ $P = 0.87$
Linyphiidae biomass	$F_{1, 80} = 24.2$ **$P < 0.0001$**	$F_{1, 80} = 3.5$ $P = 0.07$	$F_{3, 80} = 27.1$ **$P < 0.0001$**	$F_{1, 80} = 0.00$ $P = 0.95$	$F_{3, 80} = 6.7$ **$P = 0.0004$**	$F_{3, 80} = 1.5$ $P = 0.22$	$F_{3, 80} = 0.3$ $P = 0.83$
Lycosidae SR	$F_{1, 80} = 5.4$ $P = 0.0223$	$F_{1, 80} = 1.9$ $P = 0.17$	$F_{3, 80} = 86.8$ **$P < 0.0001$**	$F_{1, 80} = 0.2$ $P = 0.69$	$F_{3, 80} = 1.1$ $P = 0.35$	$F_{3, 80} = 0.6$ $P = 0.65$	$F_{3, 80} = 0.4$ $P = 0.75$
Lycosidae abundance	$F_{1, 80} = 22.8$ **$P < 0.0001$**	$F_{1, 80} = 0.1$ $P = 0.82$	$F_{3, 80} = 29.1$ **$P < 0.0001$**	$F_{1, 80} = 2.1$ $P = 0.15$	$F_{3, 80} = 0.9$ $P = 0.45$	$F_{3, 80} = 0.0$ $P = 0.9989$	$F_{3, 80} = 0.4$ $P = 0.77$
Lycosidae biomass	$F_{1, 80} = 0.0$ $P = 0.85$	$F_{1, 80} = 1.6$ $P = 0.21$	$F_{3, 80} = 31.5$ **$P < 0.0001$**	$F_{1, 80} = 2.2$ $P = 0.14$	$F_{3, 80} = 1.6$ $P = 0.20$	$F_{3, 80} = 1.5$ $P = 0.21$	$F_{3, 80} = 1.8$ $P = 0.15$

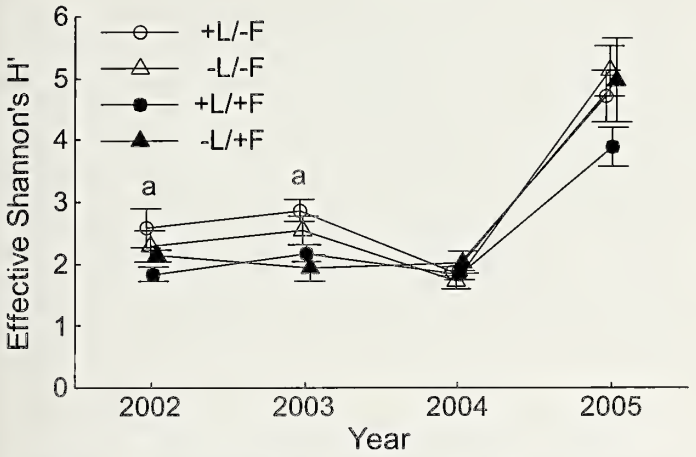


Figure 1.—Average effective Shannon's H' ($e^{H'}$) of spider species in each treatment; the letter "a" above a year denotes significance at $\alpha < 0.05$ for fertilization. Open circles (○) and "+L/−F" represent the control treatment plots of unfertilized and litter left in situ, open triangles (Δ) and "−L/−F" represent unfertilized and litter removed plots, filled circles (●) and "+L/+F" represent fertilized and litter left in situ plots, and filled triangles (▲) and "−L/+F" represent fertilized and litter removed plots.

Aggregate ecosystem-level analyses.—The NMS ordination with Araneae species richness showed clustering of plots into treatments (Fig. 6A), and the ordination axes explained 78.9% of the variance, with the first axis explaining 44.8% of the variance, and the second axis explaining 34.1% of the variance. The final stress = 6.23 with a final instability = 0.099, and results of the Monte Carlo simulation indicated that this stress was less than expected by chance ($P = 0.001$). Following Clarke (1993), a final stress between 5 and 10 was a very good ordination and did not present any real risk of misinterpretation. The first axis separated fertilized and unfertilized plots with high correlations to PAR ($r = -0.912$) and soil moisture ($r = -0.612$) in the direction of unfertilized plots, and correlations to species richness ($r = 0.347$) and percent soil organic content ($r = 0.446$) in the direction of fertilized plots, while plant litter biomass was not well correlated ($r = -0.127$). The second axis separated litter removed from litter left in situ plots with a strong correlation to plant litter biomass ($r = 0.916$) in the direction of litter left in situ plots and PAR ($r = -0.529$) in the direction of litter removed plots, but only weak or no correlations to the other three variables: spider species richness $r = -0.230$, percent soil moisture $r = -0.144$ and percent soil organic content $r = -0.034$.

Although the NMS ordination with Lycosidae did not produce a stable result, the NMS ordination with Linyphiidae species richness showed clustering of plots into treatments (Fig. 6B), and the ordination axes explained 92.9% of the variance, with the first axis explaining 71.7% of the varianee and the second axis explaining 21.2% of the variance. The final stress = 8.89 with a final instability = 0.056, and results of the Monte Carlo simulation indicated that this stress was less than expected by chance ($P = 0.002$). The first axis separated fertilized and unfertilized plots with high correlations to PAR ($r = -0.953$) and soil moisture ($r = -0.491$) in the direction of unfertilized plots, and correlations to linyphiid species richness ($r = 0.815$) and percent soil organic content ($r = 0.466$) in the direction of fertilized plots, while plant litter biomass was not well correlated ($r = 0.183$). The second axis separated litter removed from litter left in situ plots with a strong correlation to plant litter biomass ($r = 0.942$) in the direction of litter left in situ plots and weakly correlated to linyphiid species richness ($r = -0.359$) and percent soil organic content ($r = -0.297$) in the direction of litter removed plots, and no correlations to the other two variables: PAR $r = -0.039$ and percent soil moisture $r = -0.034$.

For both NMS ordinations, the separation of plots into treatment clusters was supported by MRPP (Table 4). When all four treatments were run together, the null hypothesis of no difference between treatments was rejected, with high within-group agreement and very strong separation between groups. Pairwise comparisons of treatments showed that fertilized plots, while still significantly distinct, were more similar to each other than fertilized treatment plots were to any of the unfertilized treatment plots. The same pattern existed for unfertilized plots, with strong separation of unfertilized plots, yet with lower dissimilarity than when unfertilized plots were compared to fertilized plots. As expected, the maximal differences occurred when extremes of treatments were paired, as in −L/−F vs. +L/+F, and +L/−F vs. −L/+F, indicating that "opposite" treatments significantly alter biotie and abiotic components of the local habitat.

Table 3.—Results of the GLMM for six species. Given are the F values with degrees of freedom, and the resulting P values, where bolded values indicate significance at $\alpha < 0.0012$ (after Bonferroni correction). The predictor variables are fertilization (F), litter (L), year (Y), and (*) their fully factorial interactions.

Response Variable	F	L	Y	F*L	F*Y	L*Y	F*L*Y
Pardosa moesta	$F_{1, 80} = 47.9$ **$P < 0.0001$**	$F_{1, 80} = 1.5$ $P = 0.22$	$F_{3, 80} = 7.9$ **$P = 0.0001$**	$F_{1, 80} = 0.3$ $P = 0.61$	$F_{3, 80} = 3.8$ $P = 0.0141$	$F_{3, 80} = 0.3$ $P = 0.80$	$F_{3, 80} = 0.1$ $P = 0.94$
Piratula minuta	$F_{1, 80} = 0.3$ $P = 0.59$	$F_{1, 80} = 0.6$ $P = 0.44$	$F_{3, 80} = 7.9$ **$P = 0.0001$**	$F_{1, 80} = 7.2$ $P = 0.0090$	$F_{3, 80} = 1.8$ $P = 0.14$	$F_{3, 80} = 0.9$ $P = 0.45$	$F_{3, 80} = 1.5$ $P = 0.21$
Bathyphantes pallidus	$F_{1, 80} = 26.1$ **$P < 0.0001$**	$F_{1, 80} = 4.3$ $P = 0.0422$	$F_{3, 80} = 27.2$ **$P < 0.0001$**	$F_{1, 80} = 0.2$ $P = 0.67$	$F_{3, 80} = 7.8$ **$P = 0.0001$**	$F_{3, 80} = 1.0$ $P = 0.39$	$F_{3, 80} = 0.3$ $P = 0.82$
Eridantes erigonoides	$F_{1, 80} = 0.6$ $P = 0.46$	$F_{1, 80} = 1.3$ $P = 0.26$	$F_{3, 80} = 17.3$ **$P < 0.0001$**	$F_{1, 80} = 4.3$ $P = 0.04$	$F_{3, 80} = 1.0$ $P = 0.38$	$F_{3, 80} = 1.7$ $P = 0.18$	$F_{3, 80} = 0.2$ $P = 0.88$
Collinsia plumosa	$F_{1, 80} = 8.0$ $P = 0.0059$	$F_{1, 80} = 0.1$ $P = 0.80$	$F_{3, 80} = 7.1$ **$P = 0.0003$**	$F_{1, 80} = 0.2$ $P = 0.64$	$F_{3, 80} = 3.9$ $P = 0.0114$	$F_{3, 80} = 0.2$ $P = 0.87$	$F_{3, 80} = 0.2$ $P = 0.88$
Schizocosa avida	$F_{1, 80} = 22.8$ **$P < 0.0001$**	$F_{1, 80} = 7.3$ $P = 0.0083$	$F_{3, 80} = 15.8$ **$P < 0.0001$**	$F_{1, 80} = 5.3$ $P = 0.0245$	$F_{3, 80} = 11.1$ **$P < 0.0001$**	$F_{3, 80} = 5.6$ $P = 0.0016$	$F_{3, 80} = 4.8$ $P = 0.0041$

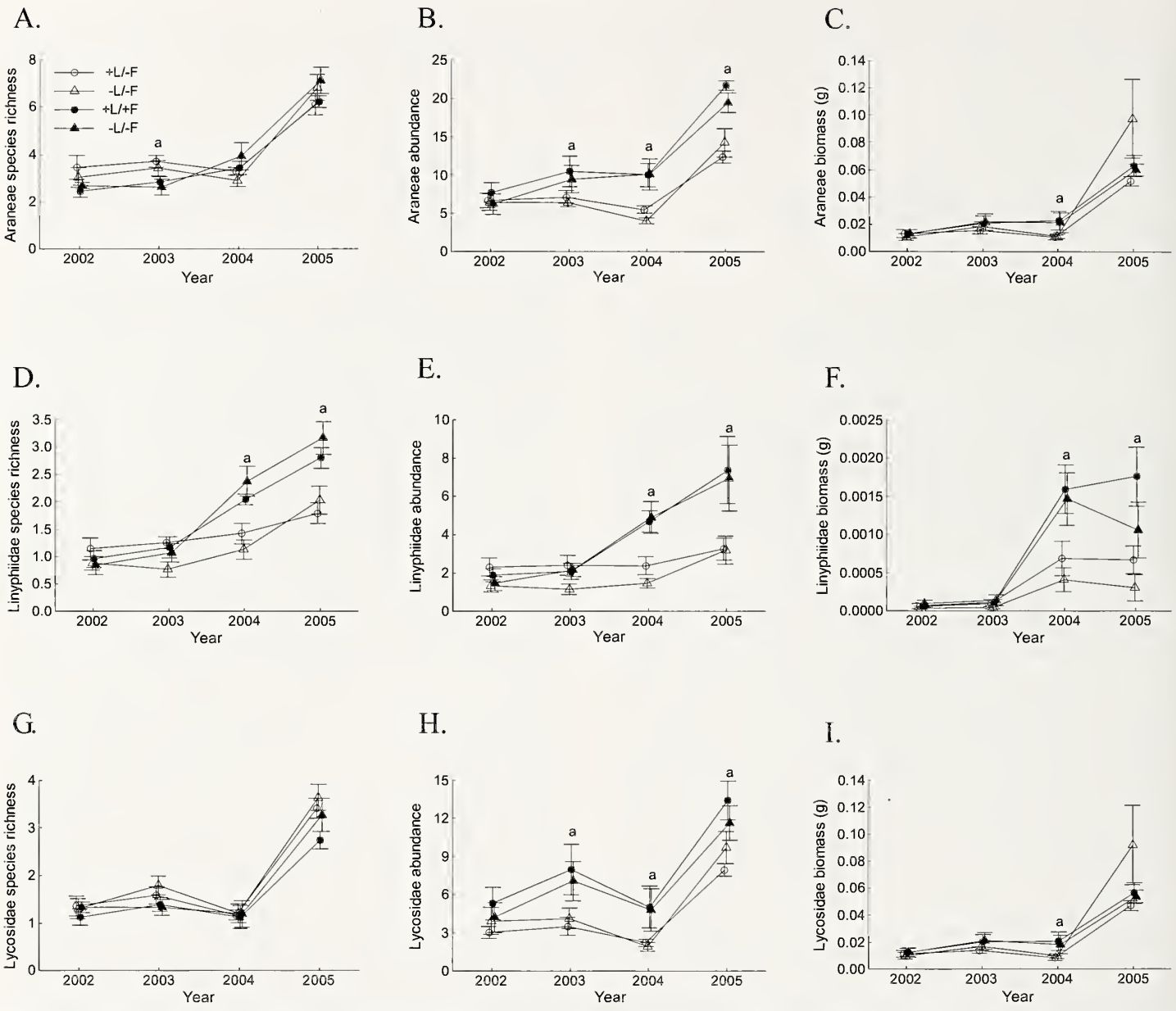


Figure 2.—Species richness, abundance, and biomass of all spiders (A–C), linyphiids (D–F), and lycosids (G–I). Definitions of symbols and abbreviations for treatments are given in Figure 1, while the letter "a" above a year denotes significance at $\alpha < 0.05$ for fertilization.

DISCUSSION

Spider abundance increased as a result of fertilization, but neither spider biomass nor spider species richness was significantly affected, and therefore our first hypothesis was not supported. This result is contrary to previous studies in which arthropod diversity in fertilized plots decreased as abundance increased (e.g., Knops et al. 1999; Haddad et al. 2000, 2001). However, our null result for the overall spider community likely resulted from of a canceling effect of the responses of the two dominant spider families, the wolf spiders (Lycosidae) and the linyphiid spiders (Linyphiidae). Wolf spider abundance was indeed significantly affected by fertilization, but wolf spider biomass and species richness were not affected. As nearly two-thirds of all spiders captured were wolf spiders, the response of this family drove the patterns found in the overall spider data, though it should be noted that our pitfall trap sampling method may have more bias towards wolf spiders due to their cursorial habit.

On the other hand, fertilization increased the abundance, biomass, and species richness of linyphiid spiders during the final two years of the study. The species richness response was completely opposite of the predictions of biodiversity-productivity theory (Haddad et al. 2000, 2001; Suding et al. 2005). This was likely a result of a bottom-up food web response to fertilization by the main linyphiid spider food source, collembolans (Harwood et al. 2001; Romero & Harwood 2010). Collembolans did not respond to the treatments despite the increased abundance of plant litter (L. B. Patrick, unpublished data), with the basal resource for the collembolan prey being the bacteria that aid in the breakdown of plant litter. Thus, while the basal resource likely increased, the primary consumer of that resource, collembolans, did not, but

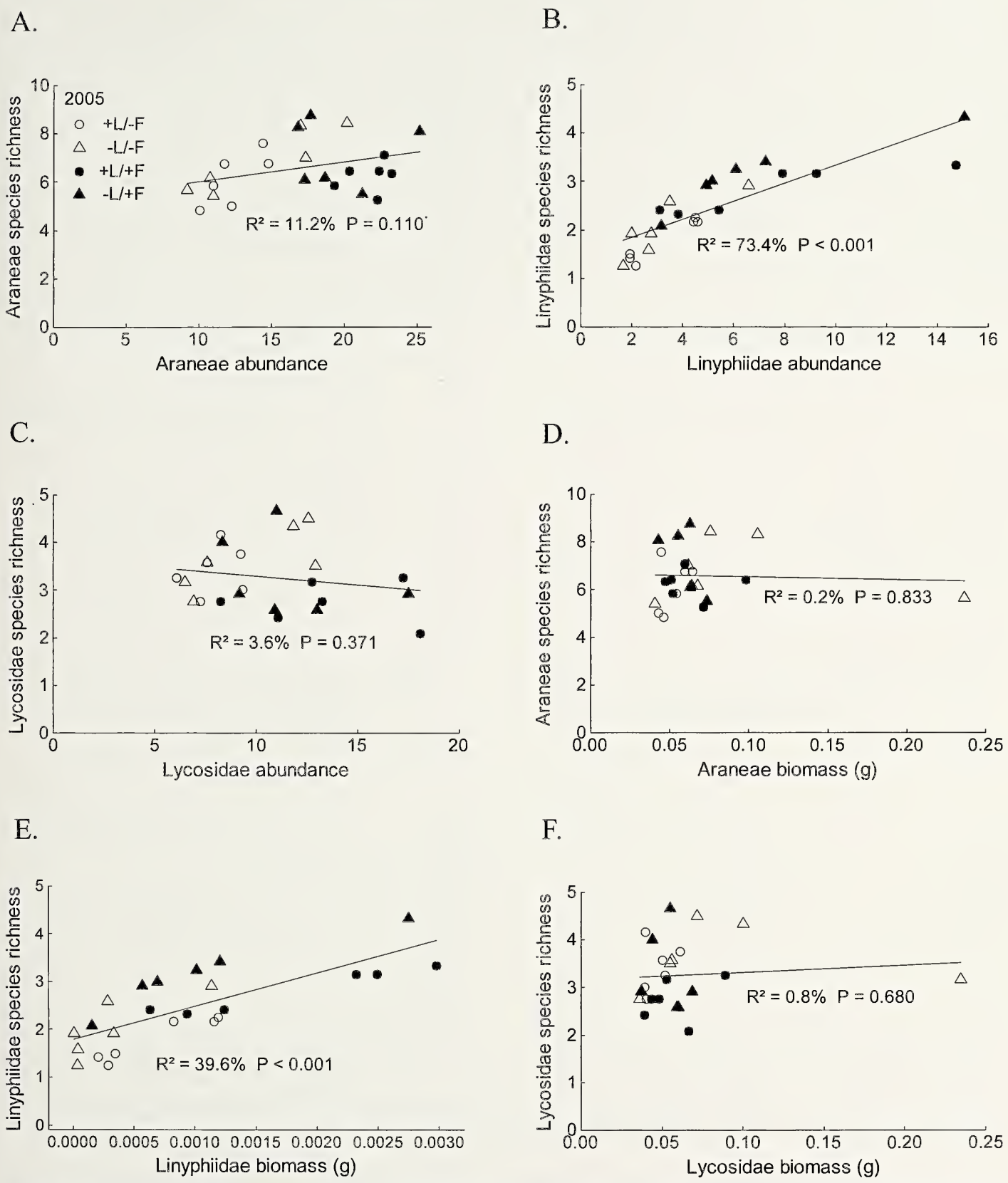


Figure 3.—Regressions of all spiders, linyphiids, and lycosids (left to right) against abundance (A–C) and biomass (D–F). Symbols are defined in Figure 1, and data presented are for 2005.

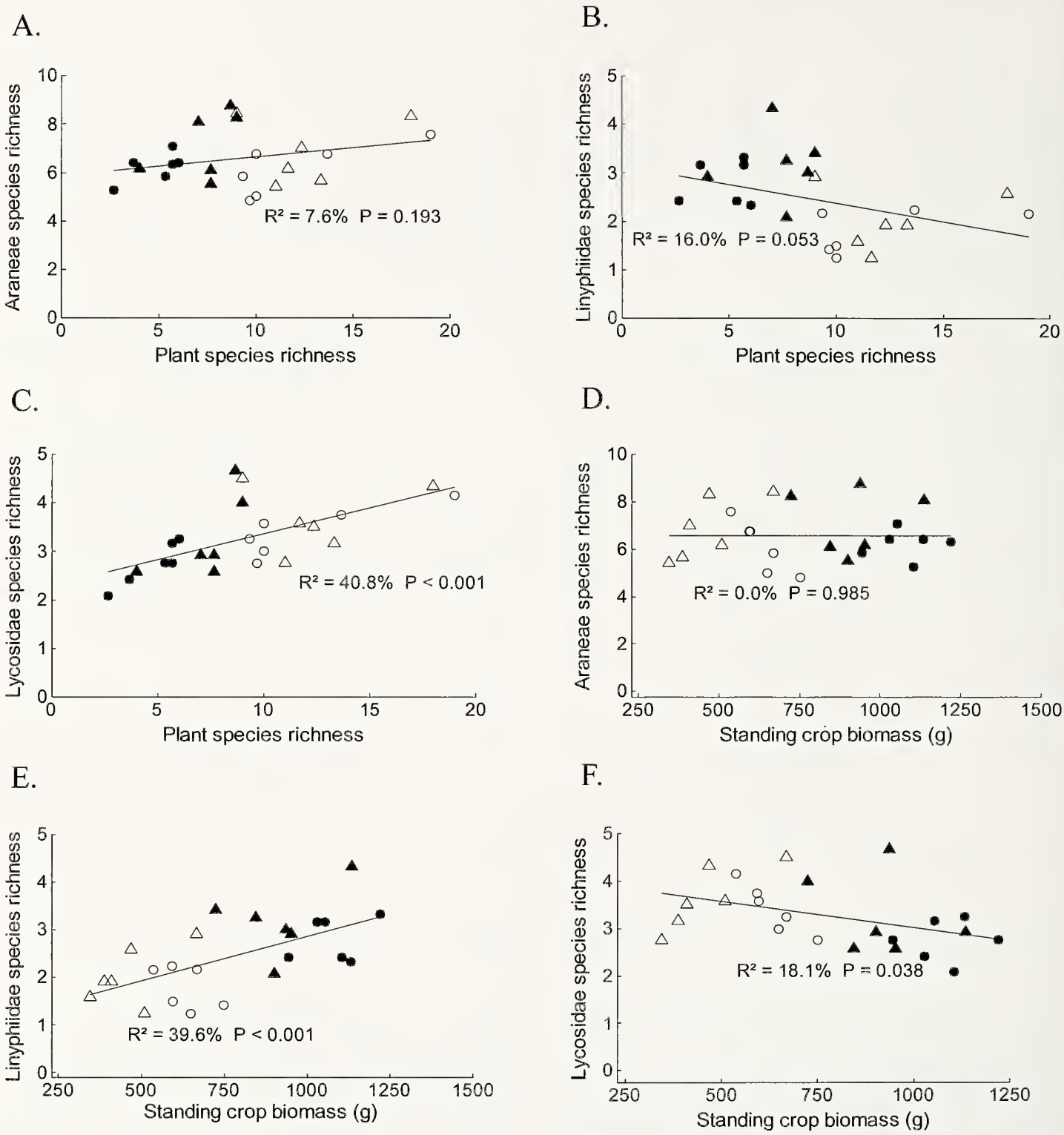
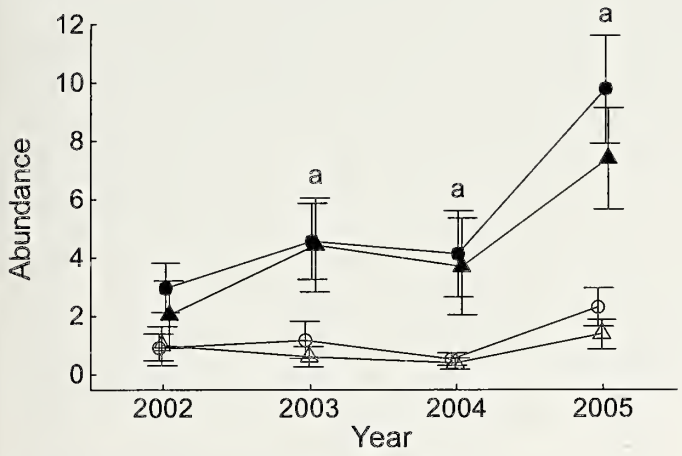


Figure 4.—Regressions of all spiders, linyphiids, and lycosids (left to right) against plant species richness (A–C) and standing crop biomass (D–F). Symbols are defined in Figure 1, and data presented are for 2005.

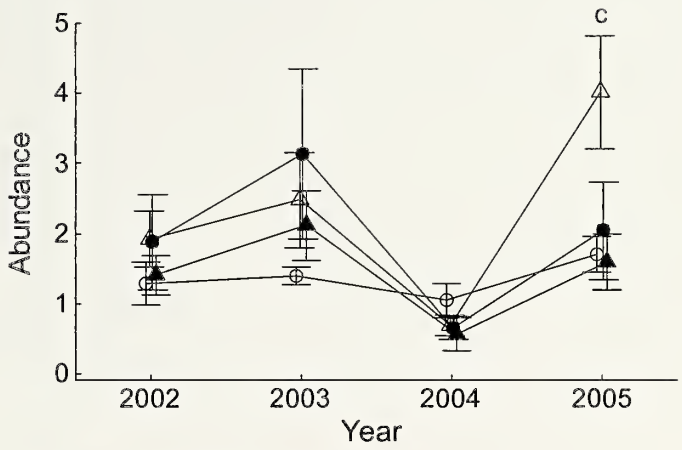
the primary collembolan predator did increase in abundance and diversity. It is therefore feasible to propose that a top-down food web effect by linyphiid spiders limited collembolan abundance, ultimately enhaneing their own abundance and diversity.

The differences in responses between wolf and linyphiid spiders are likely the results of different foraging behaviors. Spider guilds are based primarily on foraging behavior, habitat preferences and web type (Uetz et al. 1999). Based upon this classification system, wolf spiders are considered ground-running spiders, and linyphiid spiders are characterized as wandering sheet/tangle web weavers. Wolf spiders are classic epigeal wandering spiders that actively hunt for prey

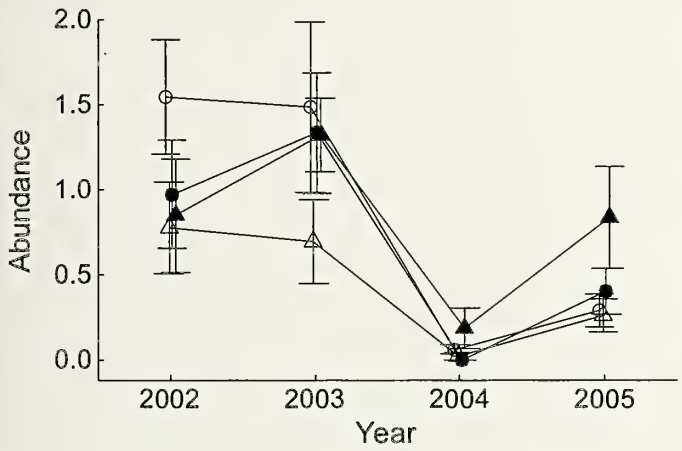
A. *Pardosa moesta*



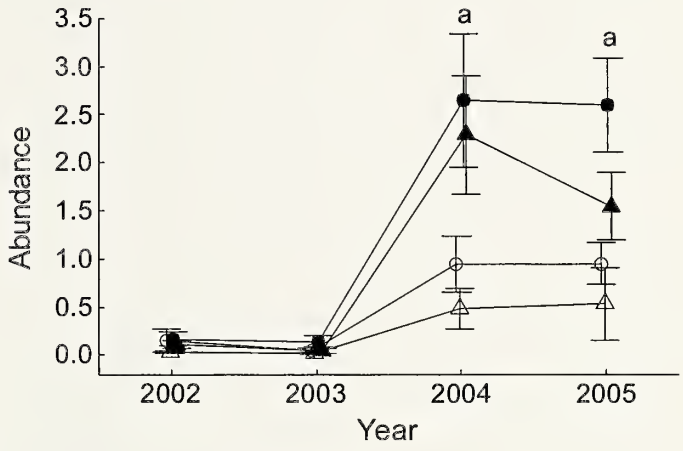
B. *Piratula minuta*



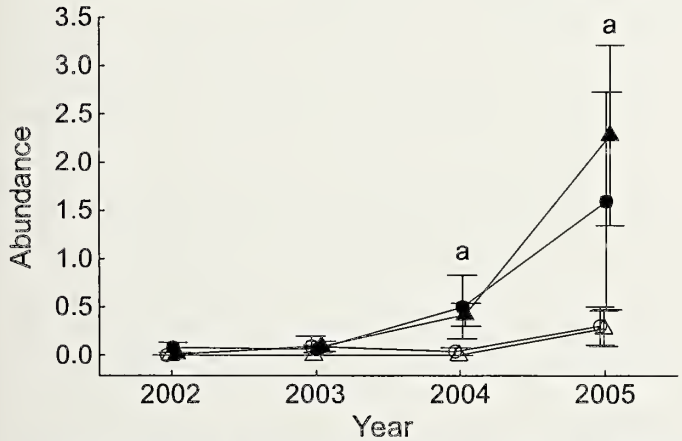
C. *Eridantes erigonoides*



D. *Bathyphantes pallidus*



E. *Collinsia plumosa*



F. *Schizocosa avida*

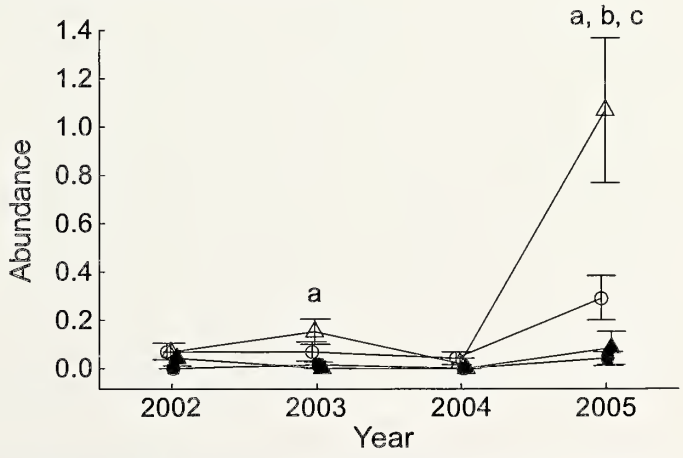


Figure 5.—Average abundance of selected species by year. Definitions of symbols and abbreviations for treatments are given in Figure 1, while the letters above each year denote significance at $\alpha < 0.05$ for "a" = fertilization, "b" = litter, and "c" = the interaction of fertilization and litter.

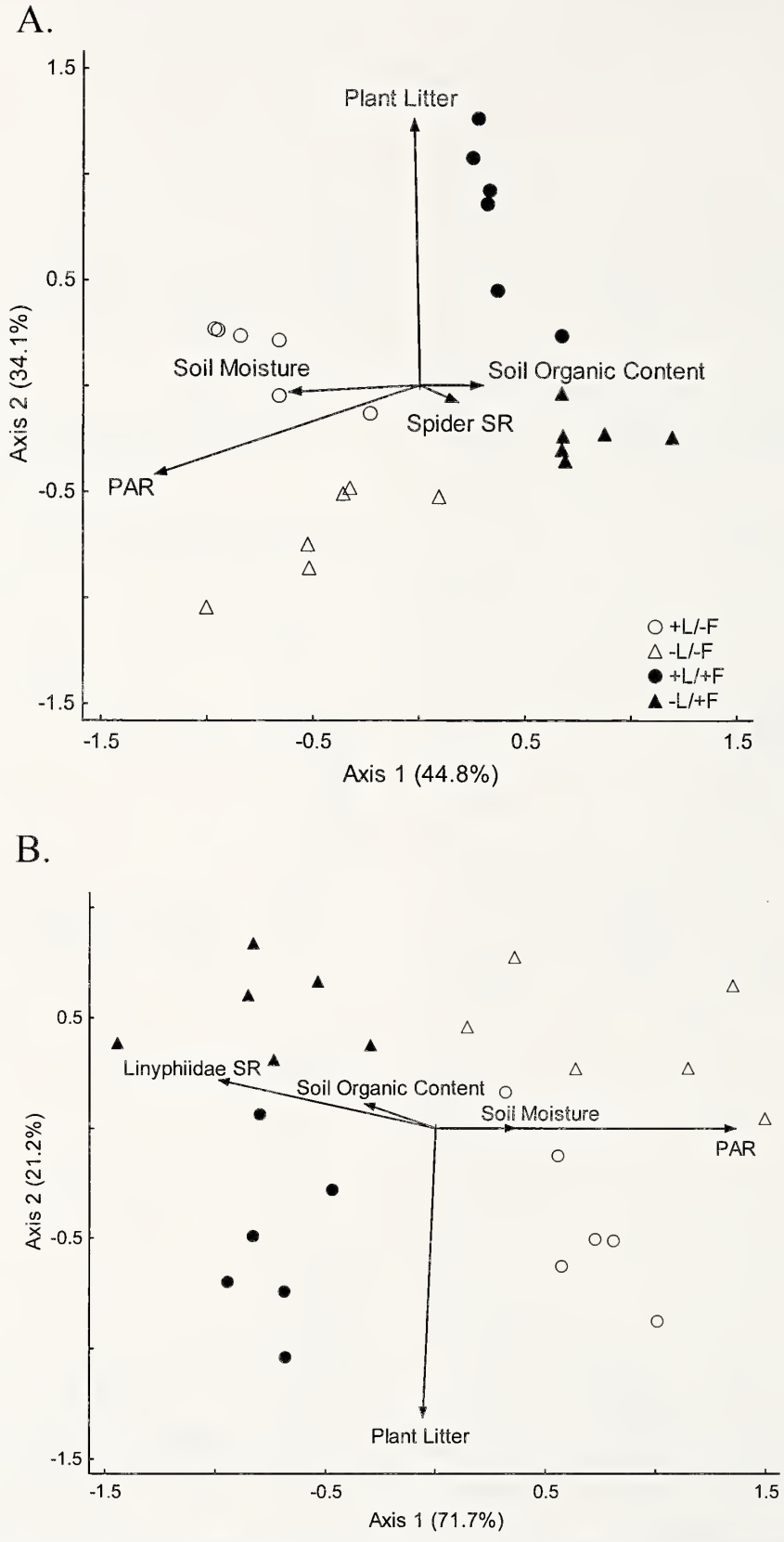


Figure 6.—Two-dimensional ordination of ecosystem-level properties from 2005 from nonmetric multidimensional scaling (NMS) using plant litter biomass, PAR, percent soil moisture, percent soil organic content, and (A) Araneae species richness or (B) Linyphiidae species richness (Lycosidae did not produce a stable result). Vectors indicate the direction and strength of correlations between axis scores and emergent properties (R^2 cutoff for joint biplot = 0.100), and ordinations are rotated to the dominant axis of fertilization. The percent of variance explained by each axis is noted next to the axis title. See Figure 1 for key to treatment symbols.

Table 4.—Results of Multi-response Permutation Procedure (MRPP) on emergent properties for 2005 to support NMS analyses (Fig. 5). *T* describes the separation between groups (dissimilarity), and *A* is the chance-corrected within-group agreement. "All" indicates all four treatments included in the MRPP, and the remainders are MRPP pairwise comparisons of treatments to assess dissimilarity (lower *T* and higher *A*). +L indicates litter left *in situ*, −L indicates litter removed, +F indicates fertilization, −F indicates no fertilization.

Groups	*T*	*A*	*P*
All Spiders			
All	−9.892	0.421	< 0.0001
+L/−F vs. −L/−F	−3.352	0.164	0.0097
+L/−F vs. +L/+F	−5.838	0.332	0.0006
+L/−F vs. −L/+F	−6.014	0.414	0.0010
−L/−F vs. +L/+F	−6.470	0.424	0.0006
−L/−F vs. −L/+F	−5.643	0.313	0.0009
+L/+F vs. −L/+F	−4.999	0.313	0.0023
Linyphiidae			
All	−10.055	0.437	< 0.0001
+L/−F vs. −L/−F	−3.236	0.159	0.0102
+L/−F vs. +L/+F	−6.395	0.373	0.0005
+L/−F vs. −L/+F	−6.445	0.442	0.0008
−L/−F vs. +L/+F	−6.501	0.435	0.0006
−L/−F vs. −L/+F	−5.842	0.338	0.0010
+L/+F vs. −L/+F	−5.188	0.284	0.0022

and are largely restricted to hunting in two-dimensional space. Thus, habitat structure (i.e., the physical structure of the surrounding environment, including plant litter and living plant material) may enhance wolf spider hunting success by providing additional hiding places for ambush hunting and for lairs (Rypstra et al. 1999; Halaj et al. 2000). However, too much habitat structure can also increase predation and intraguild predation risk through increased density responses to habitat structure while inhibiting movement (Wise 2006; Rypstra et al. 2007). These factors reduce both abundance and hunting success, thereby reducing the numbers of wolf spiders in an area. In our study, the increased habitat structure that resulted from fertilization seemed to moderately (but not significantly) reduce wolf spider species richness while increasing wolf spider abundance. However, this increased abundance was likely due to the population explosion in fertilized plots of the medium-sized wolf spider, *P. moesta* (see below).

Linyphiid spiders rely more upon webs for prey capture, sometimes maintaining and patrolling multiple webs (Uetz et al. 1999). Although these webs are generally constructed at or close to the ground level, the webs can enhance prey capture space to include a portion of a third dimension. Moreover, increased habitat structure can provide additional structure for web building (Rypstra et al. 1999). Thus, while fertilized plots significantly reduced wolf spider species richness, probably due to the enhanced habitat structure that impeded foraging ability, these plots may have provided the tiny web building linyphiid spiders the habitat structure to flourish because of the inereased structure for web building and, thus, increased prey capture rates.

A medium-sized wolf spider, *P. moesta* thrived in fertilized plots probably due to decreased intraguild predation by larger wolf spiders (e.g., *S. avida*) that became less abundant in fertilized plots. Moreover, there was increased abundance of potential prey in fertilized plots (Patrick et al. 2008b). These two factors together likely released *P. moesta* from competition and predation, resulting in an increased abundance in fertilized plots. However, these microhabitat changes likely caused the decreased abundance of *S. avida* in fertilized plots, as the increased habitat structure likely impeded this species' foraging abilities. *Piratula minuta* was one of the smallest wolf spiders captured at our site, and could have benefited from the increased habitat structure in a similar way to *P. moesta*. Although it was the second most abundant spider captured during the course of our study, it did not significantly respond to a single habitat, except for unfertilized plots with litter removed during 2005. Interestingly, *Pi. minuta* was observed on several occasions in the jaws of *P. moesta* and *S. avida*, making *Pi. minuta* a victim of intraguild predation.

Spider species richness was not significantly correlated with either plant species riehness or standing crop biomass. However, both dominant spider families responded to fertilization (by the fourth year of the study) in distinctly different ways. Wolf spiders followed predictions of current biodiversity-productivity theory, with decreased species richness associated with decreased plant species richness and increased standing crop biomass. Although fertilization increased wolf spider abundance, wolf spider species richness was correlated with plant species richness and therefore decreased as nutrient loading into the system increased.

Finally, despite documented effects of increased habitat structure on arthropod abundances and diversity (e.g., Lawton 1983; Halaj et al. 2000), particularly for spiders (e.g., Uetz 1991; Rypstra et al. 1999; Halaj et al. 2000), our results do not support our second hypothesis. Plant litter had no significant effect on spider species richness. Most studies of spider responses to plant litter have been conducted in plant monocultures in agroecosystems (e.g., Rypstra et al. 1999). These managed ecosystems tend to have much higher disturbance and more bare ground than would be expected from a grassland. Thus, increased refugia via plant litter additions to these agroecosystems would certainly provide more habitat than the existing bare ground, so it is perhaps not surprising that there have been stronger responses to plant litter in agroecosystems.

Analysis of the spider community and associated abiotic variables demonstrated strong treatment effects. These highly differentiated treatments are likely to have a strong effect on ecosystem properties (e.g., nutrient cycling, carbon sequestering), an effect likely to increase through time as the treatment plots further mature. Spiders have been shown to affect detritivore abundance (Wise et al. 1999), thereby indirectly altering nutrient cycling within the system (Chen & Wise 1999). The results of our ordinations clearly showed that our plots responded to our treatments and that the spider community affected ecosystem-level processes. The long-term implications are unknown, but it is clear that the trajectories of each treatment are significantly different and may impact ecosystem function and services. To our knowledge, this is the first time that these biotic and abiotic factors have been coupled in a multivariate ordination to explicitly determine whether they can define discrete and distinct predator

communities and their associated abiotic properties in the context of the biodiversity-productivity theory. Most previous work (e.g., Haddad et al. 2000, 2001) did not attempt to associate the invertebrate community with abiotic changes resulting from fertilization, and we know of no other studies that coupled fertilization and plant litter effects to test predictions of biodiversity-productivity theory.

The diversity and community structure of spiders and other arthropods are sensitive to plot size (Martinko et al. 2006). The large size of our experimental plots integrated important determinants of the within-plot plant communities, including spatial heterogeneity (De Boeck et al. 2006), leaching of nutrients from litter (Berendse 1998), local nutrient cycling (Hooper & Vitousek 1998) and the translocation of nutrients within clumping and clonal plants (Hutchings & Bradbury 1986), which are the primary growth forms of our dominant graminoids (Patrick et al. 2008a). These spatial factors are also important to epigeal spiders because of their vagility and their need to find suitable food; the larger plot sizes more realistically emulate natural habitat patches of varying quality and can support higher insect diversity (Martinko et al. 2006). Other studies that examined the effects of nutrient loading on arthropod communities had plot sizes ranging from 9 m^2–169 m^2 (e.g., Knops et al. 1999; Haddad et al. 2001), making our experimental plots (314 m^2) nearly twice as large—an important factor when considering the vagility of some spider species.

However, we realize that our study has some distinct differences when compared to previous work. Our use of an NPK fertilizer, as opposed to N-only fertilizer, is likely to have induced a stronger response to fertilization due to the added P and K. Nevertheless, our plant results (*see* Patrick et al. 2008a) were generally consistent with other plant studies that used NPK fertilizers (e.g., Carson & Barrett 1988; Turkington et al. 2002) and N-only fertilizers (e.g., Haddad et al. 2000; Tilman et al. 2002), which allowed us to formulate our epigeal spider hypotheses on the same bases as previous studies that investigated the responses of arthropods to nutrient loading. Further, our running definition of litter (*see* Methods) includes the vegetation mown in the previous year and not removed from litter left in situ treatment plots, potentially altering the nutritional quality of the litter relative to naturally senesced vegetation, and the physical structure of the litter as it lay after mowing (e.g., Semmartin et al. 2004). Because the timing of mowing was determined by the local township, litter from the annual mowing accumulated earlier than might normally be expected for this region of the USA. However, were the mowing to stop, the site would very quickly yield to encroaching woody vegetation typical of early secondary succession.

Our study underscores the disjunct between conventional, plant-based biodiversity-productivity theory and the animal component of the food web, partieularly epigeal predators. This portion of the food web is more closely associated with the quality of its basal resource (plant litter) than with the diversity of that resource (Cross et al. 2006; Seeber et al. 2008). This starkly contrasts with the more aerial portion of the food web that is more dependent on living plants, where specialist herbivores can be affected by plant diversity more than by plant quality. Ultimately, the loss of plant species with increased nutrient loading may result in the loss of arthropod herbivores and their specialist predators and parasites. However, the increases in diversity may be balanced by the epigeal community and its different resource base.

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Notes on the ecology and behavior of a subsocial spider *Anelosimus baeza* (Araneae: Theridiidae) in Mexico

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Abstract. Subsocial spiders are located on the continuum between solitary species and social species and are characterized by extended maternal care, some cooperation in foraging and colony activities and dispersal in order to found new colonies. In the genus *Anelosimus* (Araneae: Theridiidae), up to nine species are thought to be subsocial. One of these spiders, *A. baeza* Agnarsson (2006), is distributed across a large geographical range from Mexico to southern Brazil, and potential differences in behavior in different populations are unknown. We studied the ecology and behavior of a population of *A. baeza* in a cloud forest habitat in Mexico. We tracked the population for ten months, analyzed the degree of cooperation and the presence of associated species, and explored the settling decisions made by dispersing spiders. We show that the breeding season for *A. baeza* in Mexico differs from other populations elsewhere in South America. Using a kinematic diagram, we recorded the sequence of behaviors involved in subduing and feeding on a model prey species. Larger colonies harbored more associated species. *Anelosimus baeza* prefers to settle in locations that already contain conspecifics or silk. Our study demonstrates that *A. baeza* is a viable candidate for research into sociality in spiders and its geographical correlates.

Keywords: Araneophagy, demography, foraging, kinematic diagram, sociality

Social spiders (i.e., non-territorial permanent social or cooperative spiders: Avilés 1997) are those in which adults of the same species share a communal colony and there is cooperative prey capture and feeding. Social spiders have evolved independently several times (at least 18 times: Agnarsson 2006), most notably in unrelated families such as Theridiidae, Eresidae and Dictynidae (Avilés 1997). Recent work suggests that despite having evolved independently several times, social spiders may be evolutionary dead-ends. If sociality ultimately results in dying out of the lineage, then it is important to understand the selective pressures that drive the evolution of sociality in the first place. Among the social spiders, one of the most studied genera has been *Anelosimus* (Family Theridiidae: Agnarsson et al 2007). In this genus, 14 species have been identified as having some characteristics of sociality (Tables I and II in Lubin & Bilde 2007).

A recent reconstruction of the genus revealed a wide ranging inter- and intracontinental dispersal (Agnarsson et al. 2006). Since the geographical distribution of *Anelosimus* can span a continent, we can expect substantial variation in behavior among the different populations of the same species. *Anelosimus* spiders disperse locally at short ranges — for example, a majority of *A.* cf *jucundus* O.P.Cambridge 1896 showed a dispersal distance within a meter of origin (Powers & Avilés 2003). Though the genus *Anelosimus* is fairly widespread across the world, social *Anelosimus* are only known from the Americas, but this could be due to the relative lack of knowledge about *Anelosimus* spp. in Africa and Australasia (Agnarsson et al. 2006). Ecological and life history factors are thought to be the main drivers of the evolution of sociality in *Anelosimus*, with special emphasis on the web structure and the ability to capture large prey (Avilés 1997).

Located on the continuum between solitary spiders and social spiders, subsocial spiders are generally considered as precursors of sociality (Lubin & Bilde 2007). Subsocial spiders (non-territorial periodic social: Avilés 1997) are characterized by the following: juvenile or subadult dispersal, extended maternal care and cooperation between siblings in the natal colony (Lubin & Bilde 2007). However, since extended maternal care is also seen in other nominally solitary species (e.g. *Theridion*: Agnarsson 2004), the emphasis on designating subsocial spiders is focused on the levels of cooperative foraging (Whitehouse & Lubin 2005). Subsocial spiders are also susceptible to variations in environmental pressures such as rainfall, altitude and predator pressure (Purcell & Avilés 2008). Some other factors that could constrain subsocial *Anelosimus* are competition for colony location, competition for prey and predation from associated species (Perkins et al. 2007).

Anelosimus baeza Agnarsson (2006) is a subsocial spider found across parts of North, Central and South America (Avilés et al. 2001; Agnarsson 2006). *A. baeza* colonies are similar to solitary or small colonies of *A. eximius* Keyserling 1884 (Avilés et al. 2001). Their colonies are characterized by typical basket webs, with a capture area above and dried leaves incorporated into the colony. Since there is a lack of sex ratio bias, it has been speculated that there is outbreeding in this species; i.e., either male or female or both must leave the colony to seek mates (Agnarsson 2006). *Anelosimus baeza* is found at a range of altitudes from ca. 200 to 2500 m (Agnarsson 2006), but it is absent below 600 m in tropical rainforest (see Purcell & Avilés 2008).

Although there have been some studies on prey size and abundance, and environmental effects of predation pressure on colony survival in Ecuadorian populations of *A. baeza* (Powers & Avilés 2007; Purcell & Aviles 2008), little is known about the details of behavior seen during prey capture, preferences for founding colonies and how environmental factors affect the breeding season in other populations. Furthermore, *A. baeza* may show extreme variation in social behavior (L. Aviles, pers. comm. cited by Agnarsson 2006). Thus, basic details of ecology and behavior are needed from

different populations in order to come to a better understanding of subsociality in this species. Therefore, we designed a baseline study touching on several aspects of elemental ecology and behavior of *A. baeza* in Mexico. More specifically, we sought to determine the phenological pattern of this species. We recorded the presence of associated species (other spiders and insects) in the colonies. We studied foraging behavior in field and laboratory conditions to determine the level of cooperation between individuals. And, finally, we studied the settling decisions made by dispersing females in a greenhouse experiment.

METHODS

Study species and site.—A natural population of *A. baeza* colonies was surveyed in the Francisco Javier Clavijero Botanical Gardens, Xalapa, Mexico (19.514132°N, 96.936129°W; altitude: 1400 m). The colonies were found on the extremities of several trees. Observations on prey capture were made at the invertebrate biology laboratory in INBIOTECA, Universidad Veracruzana, Xalapa, Mexico. Spiders for the experiments were collected from trees in and around Xalapa.

Population structure.—Thirty-one colonies occupied by adult females and juveniles or females with egg sacs were marked with tags and surveyed twice a month from September 2010 to July 2011. We measured the colony volume (length × breadth × depth in cm) and recorded the number of individuals. In case of colony failure, we surveyed new ones. We recorded individuals in three categories: adult males, adult females and juveniles. The physical condition (hereafter 'status') of the colonies was scored by a single observer and separated into three categories: 1) webs with substantial damage and detritus; 2) webs with moderate damage and 3) webs with little or no damage and fresh appearance of the threads. These scores were later averaged over colonies and regressed against time elapsed since the beginning of monitoring. We analyzed the relationship between colony size (volume) and number of spiders with an ANCOVA, with date and colony number as covariates.

Associated species.—We recorded the number and presence of other associated organisms (i.e., other spiders and insects) in the colonies. Individuals were assigned to morphospecies. The Shannon-Wiener index, $H' = -\sum p_i * \log(p_i)$, where p_i is the proportion of the i^{th} species (Magurran 2004), was calculated using the software Diversity (Version 1.6) to determine the diversity of associated species for each colony. We analyzed the relationship between colony size and diversity of associated species with a linear regression.

Cooperation during foraging.—Prey capture activities from eleven different colonies were observed under field conditions. As prey, a single Mexican fruit fly, *Anastrepha ludens* (Diptera: Tephritidae), was placed in the colony, the number of spiders participating in the capture was registered, and the total number of spiders feeding on the fly was recorded an hour later. This procedure was carried out 11 times (i.e., 11 colonies) between 11:00 and 16:00.

Fourteen colonies were collected from the field by removing the whole branch and placing it in a plastic container. Colonies were kept in the containers in the laboratory for 24 hours for acclimatization. Colonies were then removed from the container and clamped into position for filming. *Anastrepha ludens* flies were placed in the webs, and foraging behavior was recorded with a digital camera (Sony DSC-HX1). Subsequently, the video recordings were analyzed with the event-recording software, Annotation (Version 1.0), to create a list of common behaviors observed from two spiders per colony during prey capture. We determined the transitional probabilities of the behaviors and constructed a kinematic diagram showing the most frequent transitions.

Settling decisions.—To determine the preference of dispersing spiders to settle on different substrates, we ran an additional experiment inside a small greenhouse (10 × 5 m). Three substrates for settlement were provided the dispersing spiders: (1) colonies consisting of web and spiders (WS, n = 6), (2) webs with no spiders in it (W, n = 6) and (3) a single branch free of web and spiders (C, n = 6). These settling substrates (separated by approximately 30 cm) were linked together in a grid (270 × 90 cm) with cotton thread. The order of the substrates was randomized. Spiders previously collected were held for one day, and marked with non-toxic paint on the abdomen with a fine paintbrush. The marked spiders (n = 30; six individuals per day for five days) were then released onto the grid along one edge at a distance of 40 cm from each other and left there for 24 hours. The location of marked spiders was registered after 24 hours. Settling preferences were analyzed with a chi-square test of goodness of fit.

Statistical Analyses.—All data were checked for normality before analysis. We used the statistical software GraphPad Prism (version 5) and JMP (Version 9) for all analyses.

RESULTS

Population structure.—*Anelosimus baeza* colonies were mostly found on the extremities of trees such as *Podocarpus* sp. (27% of colonies recorded) and *Citrus* spp. (39%), and a few colonies were also recorded on trees such as *Talauma mexicana* (17%), *Schefflera* sp. (7%), *Ficus* sp. (5%), as well as bamboo (5%). Especially in the citrus trees, colonies were sometimes located very close to each other on adjacent branches, and occasionally we observed silken connections between the colonies. Web construction activity was seen intermittently throughout the day. The webs of the colonies followed the typical pattern of *Anelosimus* webs with a basket or a sheet at the base, usually containing dry leaves and with capture threads extending upwards in a roughly pyramidal shape. Occasionally, small colonies (probably recently dispersed individuals) would build on a single leaf or a few leaves. Larger colonies had more individuals (ANCOVA: $F_{1,528}$ = 217.7, $P < 0.0001$). We recorded 10 instances where the colonies became reduced in volume until there were no individuals left. Furthermore, we also observed two instances where previously defunct colonies were subsequently recolonized.

We monitored *A. baeza* colonies (n = 41) for 10 months and observed a decline in the number of individuals in the course of the year. Fig. 1 shows the decline in juveniles as the season changes. Females are present throughout the year with the possible exception of January, whereas males begin to appear in February and last till June. The period between February and June (possibly until July) is the breeding period, with juveniles appearing in August and September. We recorded

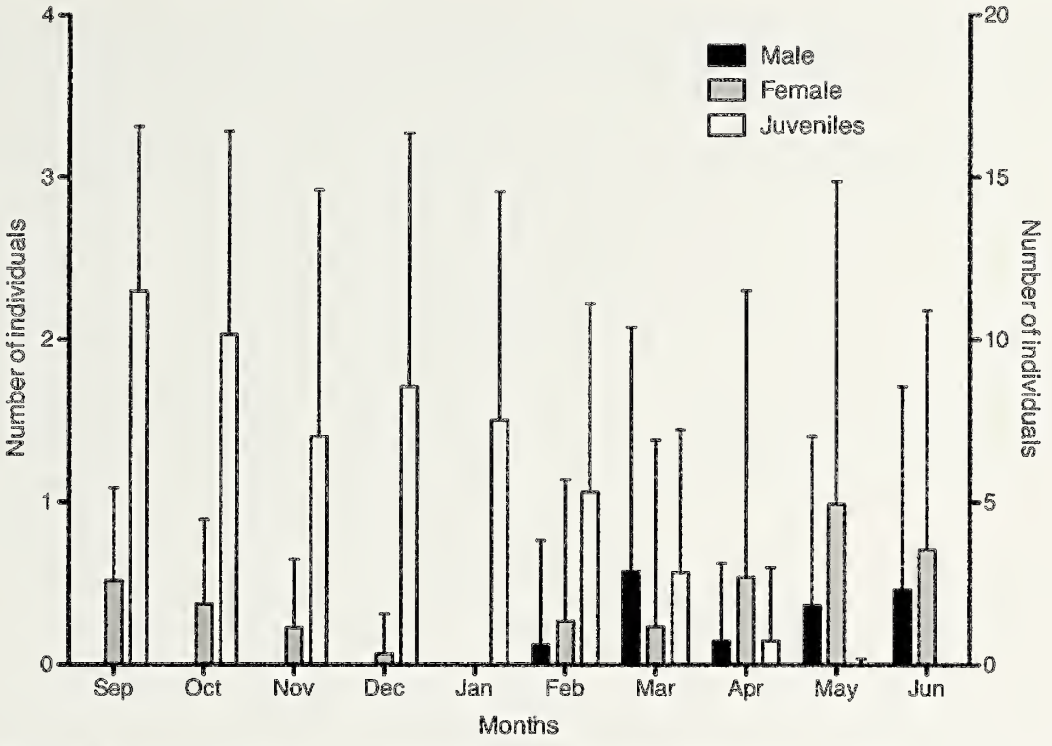

Figure 1.—Phenology of *A. baeza* colonies over a period of ten months, showing abundance of adult males, females and juveniles. The secondary y-axis gives the number of juveniles.

more than one adult female in the same colony (but separated spatially), suggesting some level of tolerance toward conspecific females. The average status of the colony declined over time (linear regression, $R^2 = 0.9$, $n = 15$, $P < 0.0001$).

Associated species.—Colonies harbored different heterospecific species, including potential prey, that were sheltered in the colonies, though not in direct contact with the capture web (Table 1). Diversity of associated species significantly increased with the average volume of the colony (Fig. 2; linear regression, $R^2 = 0.18$, $n = 40$, $P < 0.01$).

Cooperation in foraging.—*Anelosimus baeza* shows broad cooperation in prey capture: both males and females, as well as juveniles, attacked the prey together. Experimentally placed model prey (*Anastrepha ludens*) were attacked collectively by most members of the colony in both field and laboratory conditions. In field conditions, we estimated that approximately 60% of the spiders participated in the hunt, and there was a significant positive relationship between the number of spiders attacking and the number of spiders in the colony ($R^2 = 0.48$, $n = 11$, $P = 0.0176$). From preliminary observations, we identified and codified a list of units of behavior observed during foraging. The most frequent transitions were Retreat from prey → Approach prey, Silk throwing → Bite prey, and Retreat from conspecific → Stand still (Fig. 3).

Even though the spiders cooperate in hunting prey, we observed frequent fights among conspecifics. Fights were most common between females. The process from approach prey to feeding is very dynamic and involves a series of steps (Fig. 3). We did not observe any synchronization in movements between colony members or periodic immobility as seen in *A. eximius* (sensu Krafft and Pasquet 1991; see discussion).

Settling decisions.—Spiders significantly preferred to settle in locations already containing spiders (50%, 15 individuals) and webs, followed by webs only (13.3%, 4 individuals) ($\chi^2 = 14$, $df = 2$, $P < 0.001$). Control branches (without spiders or webs) were very rarely (6.6%, 2 individuals) chosen as substrates. Thirty per cent (9 individuals) of the spiders disappeared and were not recovered.

DISCUSSION

Our investigation of the basic ecology and behavior of *A. baeza* showed that the breeding season occurs from February to June. There is a significant relationship between the size of the colony and the presence of associated species, suggesting that as the colony grows larger, more niches are available for heterospecific species and also there is an increase in potential predation pressure. Most colonies contain a maximum of two adult females, which is similar to another subsocial spider, *A. vierae* Agnarsson 2012 (ex *cf studious*: Viera et al 2007; Agnarsson 2012). Our analysis of the foraging behavior of the species suggests that though there is cooperative hunting, it is fairly individualistic with frequent aggressive interactions between conspecifics, and as such can be described as 'hunting in the presence of a companion' (sensu Whitehouse & Lubin 2005). Females preferred to settle in locations with pre-existing colonies rather than establishing a new colony in a vacant space, a common behavior seen in other colonial araneids (e.g., Rao & Lubin 2010).

Prey size and abundance were previously studied in an Ecuadorian population of *A. baeza* (Powers & Avilés 2007). This study showed that prey capture rate is low, and *A. baeza* captures smaller prey than social *Anelosimus*. Larger prey

Table 1.—Other species associated with *A. baeza* colonies.

Taxa	Number of morphospecies
Arachnids	
Clubionidae	2
Tetragnathidae	1
Salticidae	3
Theridiidae	4
Thomisidae	1
Mimetidae	1
Araneidae	1
Opiliones	1
Insects	
Coccidae	2
Dermaptera	1
Lepidoptera	1

(relative size to the spider) were captured early in the season than later, suggesting that the presence of many juveniles aids in the capture of larger prey. Purcell et al. (2008) carried out a transplant experiment to test the effect of different levels of altitude, rainfall and predation pressure on colony survival in *A. baeza* in Ecuador. They showed that the colonies that were transplanted to lower altitudes (from 2100 m to 1000 m and 400 m) failed faster than ones transplanted to higher altitudes Furthermore they showed that rainfall intensity affected the number of spiders remaining in the colony. Colonies that were protected from the rain built significantly more web material than colonies that were exposed. Nentwig & Christenson (1986) studied the natural history of *A. jucundus* in Panama. However, a recent revision of *Anelosimus* suggested that the species in Panama is probably *A. baeza* and not *A. jucundus* (Agnarsson 2006). Accordingly, the Panamanian species' colonies can contain more than one adult female in the web and possibly non-cooperative prey capture (Nentwig & Christenson 1986), which differs from *A. baeza* in Ecuador.

Anelosimus baeza has been suggested to have a large variation in social behavior across populations (L. Aviles in Agnarsson 2006), but social polymorphism (sensu Riechert & Jones 2008) needs to be tested. If the population of *A. jucundus* in Panama is *A. baeza*, as suggested by Agnarsson (2006), then there are substantial differences between the two populations, despite their relative proximity. In Panama Nentwig & Christenson (1986) found up to six adult females in a single colony, but we never found more than two. Furthermore, there was no cooperative feeding between the females, whereas in this study we observed cooperative hunting. Nentwig & Christenson (1986) also base their speculation of lack of cooperation on the fact that adult females seemed to be

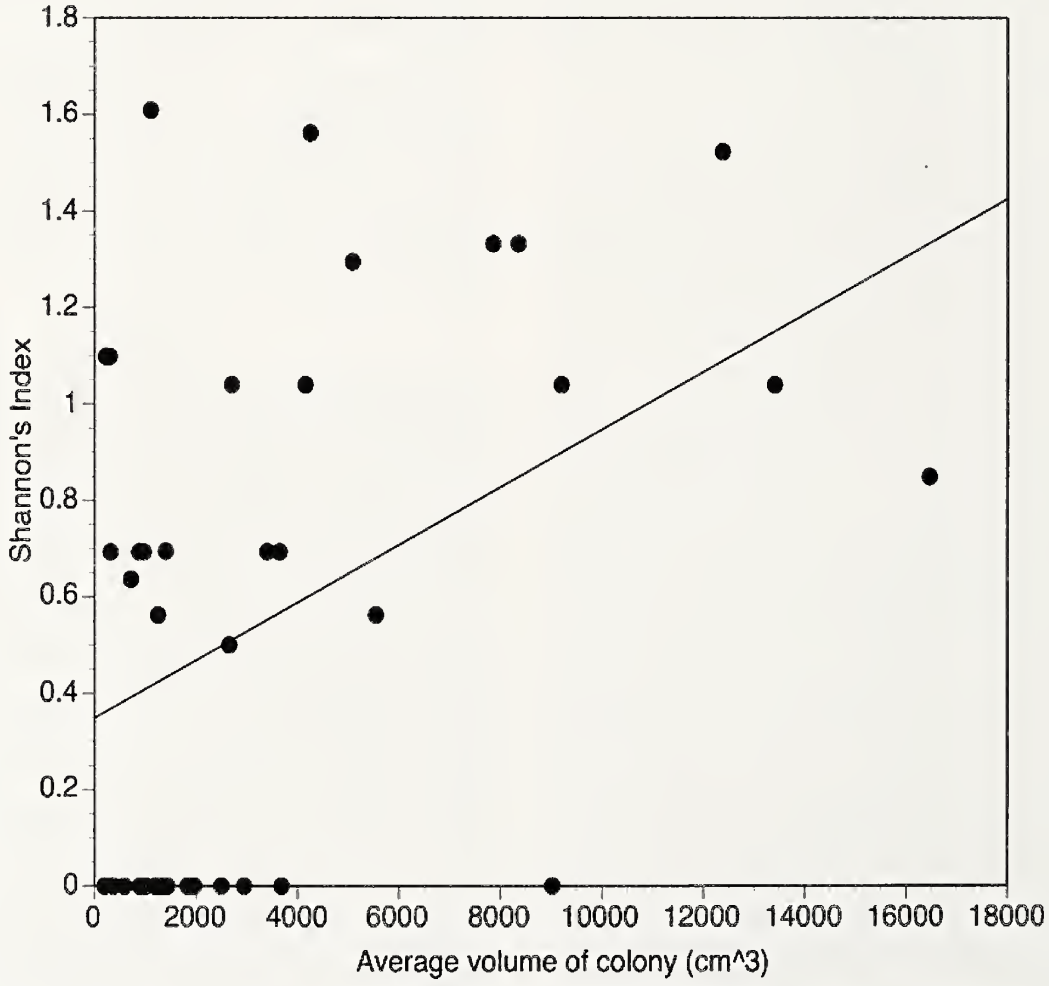

Figure 2.—Diversity of associated species found in *A. baeza* colonies increased with the volume of the colony ($R^2 = 0.18$, $n = 40$, $P < 0.01$).

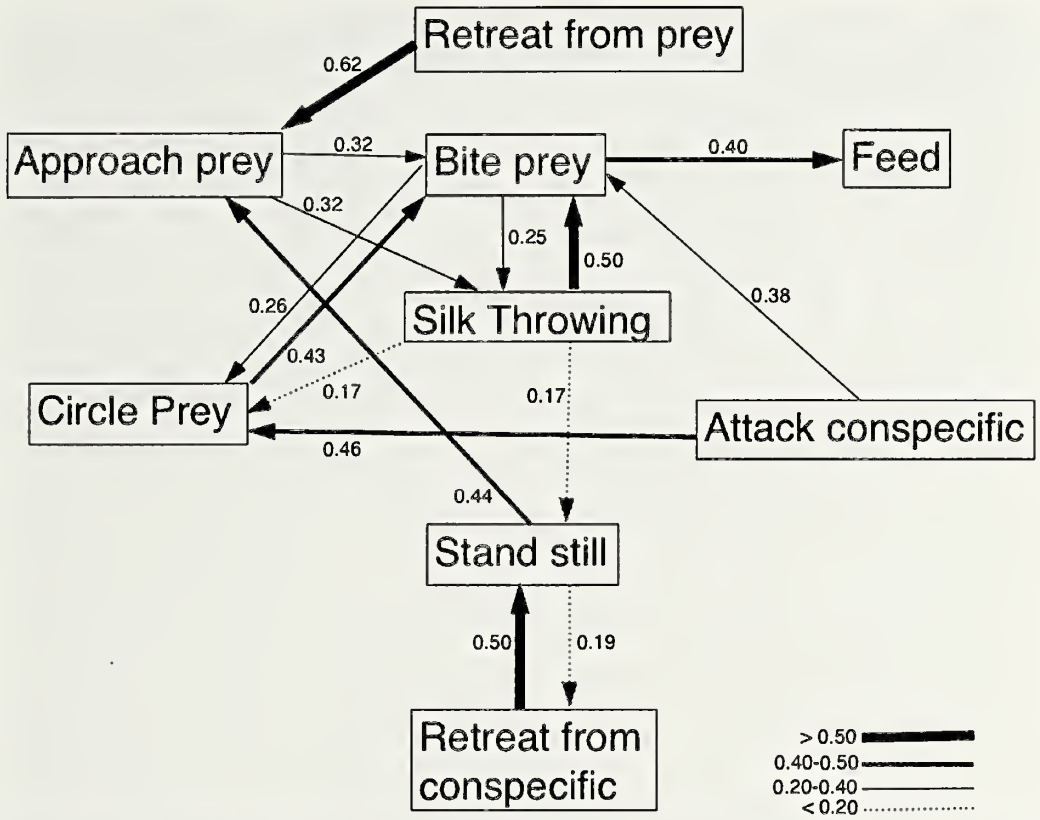

Figure 3.—Kinematic diagram showing the transitional probabilities of common behaviors during foraging. Less common behaviors, namely Move prey and Cutting threads are not shown.

spatially separated from one another, as they were located under different leaves of the same Compositaceae plant. In our study, all colonies were located in the extremities of trees, with no distinct stratification.

Anelosimus baeza in Mexico also seems to follow a different phenology than populations in Eeuador and Panama. For example, egg sacs are seen from December to February in Ecuador (Powers & Avilés 2007) and from February to April in Panama (Nentwig & Christenson 1986), whereas in this study egg sacs were observed as early as October and throughout December in Mexico. Variation in phenology may be related to differences between populations in altitude, latitude and rainfall.

If there are differences in social behavior, there may be population level differences in foraging behavior as well. The kinematic diagram shows that biting the prey does not always lead to feeding on the prey. Between colonies, individuals show considerable variation in their behavior, attacking other conspecifics and circling prey. These aggressive interactions suggest that although the spiders are hunting the same prey, they are not necessarily hunting together. This interpretation is further strengthened by the fact that not all individuals participate in the hunt. In any cooperative hunting species, there are bound to be a few free riders, resulting in hunting success decreasing with group size after group size reaches some optimal level (e.g., in wolves: MacNulty et al. 2011). We noted that silk throwing, wherein the spider quickly drew silk from the spinnerets and flung it at the prey in order to immobilize it, was frequently followed by biting the prey. This suggests that cooperation in hunting is most obvious at this stage; i.e., immobilization of the prey. Therefore larger prey should lead to more cooperation, as seen in *A. eximius* (Souza et al. 2007). Feeding occurs directly on the prey in *A. baeza*, unlike in social spiders such as *Stegodyphus sarasinorum* Karsch 1891, where parts of the prey are transported back to the central parts of the colony (D. Rao pers. obs.).

We did not observe any synchronization between individuals as seen in *A. eximius* (Krafft & Pasquet 1991), where spiders exhibit periodic states of motion and immobility. Kraft & Pasquet (1991) suggested that this pattern of synchronization and stillness enhances prey localization by eliminating potentially confounding vibrations generated by the spiders themselves. However, *A. baeza* webs are very small compared to those of *A. eximius*, and hence there may not be a need to develop specific patterns of intra-individual communication. Furthermore, the number of spiders that attack a prey is determined by the size of the prey (Souza et al. 2007). In our study, we used a single model prey species and thus did not determine differences in levels of cooperation due to prey size.

Since the colonies of *Anelosimus* spiders accumulate dead leaves and are fairly stable in time and space, they create a new microhabitat that is subsequently exploited by other organisms (Viera et al. 2007). Interest in associated species has focused on either kleptoparasitic spiders (Nentwig & Christenson 1986) or araneophagic spiders (Perkins et al. 2007). Araenophagic predators of *Anelosimus* have been recorded from the following spider families: Anyphaenidae, Agelenidae, Salticidae, Pholcidae (Jackson & Rowe 1987; Jackson 2000; Perkins et al. 2007; Viera et al. 2007). We also observed kleptoparasitic and araneophagic spiders, and our results

Figure 4.—First record of predation of *A. baeza* by an araneophagic spider (Araneae: Mimetidae).

suggest that as colony size increases, *A. baeza* has the potential to harbor more species. Furthermore, the probability of colony failure is also linked to the number of associated species, but it is unclear from our study whether poorly defended colonies are invaded more often or whether invasion causes the colony to fail. A similar finding was reported in *A. studiosus*, where there was a close correspondence between the rate of loss of colonies over time and the association rate of anyphaenids and agelenids (Perkins et al. 2007).

Furthermore, we observed direct predation by a mimetid species (Fig. 4) on *Anelosimus* for the first time. We suggest that, in accordance with Purcell and Aviles' findings (Purcell & Avilés 2008), salticids primarily use *Anelosimus* colonies as a refuge rather than for predation, though they might capture *Anelosimus* facultatively. We also recorded a few inseet species within the colony (Table 1), but since they did not come into contact with the capture threads, they may not be treated as prey.

Predation by ants is considered to be a major factor influeneing the distribution of subsocial *Anelosimus* in Ecuador, as there was a greater abundance of ants in areas where the relatively small colonies of *A. baeza* suffered colony failure (Purcell & Avilés 2008). We did not note any significant incidence of ants in the colonies in our study site.

Dispersing *Anelosimus* tend to settle very close to the 'natal' colony (e.g., *A. jucundus*: Powers & Avilés 2003). In the present study we observed, but did not measure, short inter-colony distances. We also observed silken threads eonnecting closely spaced colonies, and these connections disappeared after heavy rains, only to reappear later, similar to that seen in *A. vierae* (Viera et al 2007). The pattern of joining and disconnecting colonies is reminiscent of fission-fusion dynamics seen in other cooperative species and suggests that temporary breakdown of connection may be better for continued survival of the colony than a permanent disconnect between areas of the colony (Kerth 2010). These observations are in concordance with our experiments with settling decisions, where spiders preferred to settle in pre-existing colonies. This preference may because (1) spiders show high levels of sericophily, (2) spiders treat the presence of a pre-existent colony as an indication that the site is profitable, (3) it is a strategy to avoid predation pressure due to traveling or (4) spiders avoid lost opportunity costs by settling in proven sites (Lubin et al. 1993; Jakob et al. 2001). Sericophily may be a general predisposition in spiders across different levels of sociality, since a similar pattern was seen in a eolonial spider *Cyrtophora citricola* Forsskål 1775 (Rao & Lubin 2010).

A. baeza is a continent-spanning subsocial spider and as such is a promising candidate for testing different hypotheses ranging from the evolution of sociality to the influence of geography on behavior. Our study represents a baseline view of several components of the ecology and behavior of this species. Further research will focus on comparative parallel experiments on widely separated populations.

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Behavioral analysis of the interaction between the spitting spider *Scytodes globula* (Araneae: Scytodidae) and the harvestman *Discocyrtus invalidus* (Opiliones: Gonyleptidae)

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Abstract. Spitting spiders (Scytodidae) have a distinct predatory strategy in which they eject a sticky secretion from their cheliceral fangs to immobilize prey. This behavior could potentially allow the spider not only to avoid defensive secretions but also to bite specific vulnerable spots of a potential prey such as a harvestman. We used an ethogram, a fluxogram and an experiment to analyze the interaction between the harvestman *Discocyrtus invalidus* Piza 1938 (Arachnida: Opiliones) and the syntopic spider *Scytodes globula* (Nicolet 1849) (Arachnida: Araneae). These spiders, while readily taking crickets as prey, seldom spat at and never bit the harvestmen, which apparently did not exude repugnatorial secretions. We therefore tested, by clogging the glands and using appropriate controls, whether non-visible amounts of secretions could cause the rejection, but the harvestmen were still refused. This is the first detailed and quantified description of an interaction between a spitting spider and a harvestman. The general conclusions are that *S. globula* avoids preying on *D. invalidus*, *S. globula* behaves differently when attacking harvestmen and crickets and the scent gland secretions of *D. invalidus* do not play a direct role in this predator-prey interaction.

Keywords: Chemical defense, foraging, Laniatores, prey capture, scent gland

Spitting spiders (Arachnida: Araneae) are unique among the more than 40,000 species of spiders in the way they capture prey. They spit a sticky secretion produced by cheliceral glands and extruded by their cheliceral fangs. This secretion is a mixture of glue, venom and silk (Suter & Stratton 2009; but see Clements & Li 2005) and is used to immobilize a wide variety of arthropods used as prey (Nentwig 1985; Li et al. 1999).

Potential prey items for spitting spiders are harvestmen (Arachnida: Opiliones) in the suborder Laniatores. Harvestmen are usually nocturnal and have poor eyesight (Willemart et al. 2009) and thus do not detect predators such as spiders visually. They have a combination of defenses that includes the use of chemicals, specifically an exudation of droplets of repugnatorial secretions from the scent glands located dorsolaterally on the prosoma; a heavy armature covering most of the body; and physical retaliation such as pinching with pedipalps, chelicerae, and spined legs (Gnaspini & Hara 2007; Pomini et al. 2010; Souza & Willemart 2011). Some harvestman species are preyed upon by some spiders, but others are rejected (Gnaspini & Hara 2007).

We hypothesized that spitting spiders could overcome the defenses of such harvestmen (1) by spitting from a distance instead of biting so that they would not come into contact with the harvestmen's repugnatorial secretions and (2) by spitting their viscous secretion, the spiders would limit the harvestmen's ability to move, allowing the spiders to bite specific vulnerable areas (i.e., where not protected by the heavy armature). Though spitting spiders have a delicate body and weak chelicerae, some laniatorid harvestmen like *Discocyrtus invalidus* Piza 1938 (Laniatores: Gonyleptidae) are often eaten by the syntopic recluse spider *Loxosceles* sp. (Fischer et al. 2006; Willemart & Souza pers. observ.), a spider of small body size and weak chelicerae similar to *Scytodes*. Moreover, in the field, spitting spiders have been observed eating the laniatorid harvestman *Mischonyx cuspidatus* (Roewer 1913), which is very similar to *D. invalidus* in size (Mestre & Pinto-da-Rocha 2004).

Therefore, we studied the interaction between the spitting spider *Scytodes globula* (Nicolet 1849) (Scytodidae) and the syntopic harvestman *D. invalidus*. Both occur in the same microhabitat, using dead palm fronds on soil and fallen trunks as shelter during the day and their surroundings at night as foraging areas. No previous studies have been conducted on the interaction between these two species, so it was unknown whether or not the spider preyed upon or rejected this harvestman species. Based on our hypotheses, we first predicted a similar mortality rate of harvestmen and crickets when paired with the spiders. However, our first experiment revealed that the spiders attacked crickets significantly more readily, so we conducted an investigation as to why the spiders rejected these harvestmen. By performing a careful descriptive analysis, we noticed that the spiders behaved differently when interacting with harvestmen than with crickets. We did not detect the release of defensive secretions from the scent glands of the prey. Consequently, we experimentally tested the hypothesis that harvestmen were protected by the release of small amounts of secretions not visible to the human eye (see Machado et al. 2005 for further discussion), but found no evidence of such.

METHODS

Species studied.—The harvestman *Discocyrtus invalidus* is found in tropical rainforests in southeastern Brazil, State of São Paulo, where it hides under logs and dead palm fronds during the day and wanders on tree trunks, on the ground or on bushes at night. When we insistently disturbed individuals of this species, they released conspicuous droplets of secretion from the scent glands, the openings of which can be seen with the naked eye. The defense secretion of this species is

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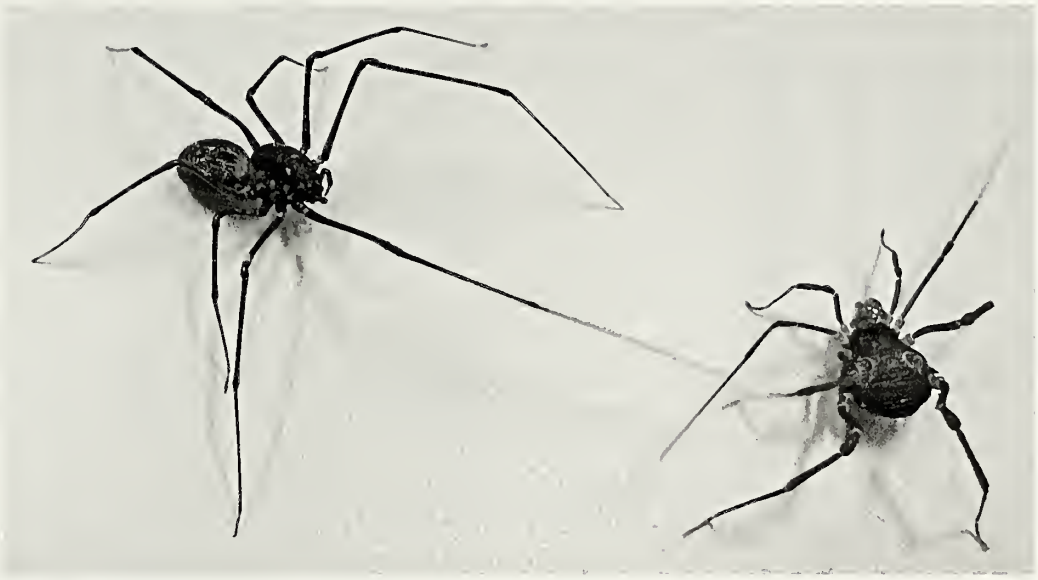

Figure 1.—*Scytodes globula* displaying "approach" and "contact leg" (see Table 1) with the harvestman *Discocyrtus invalidus* (~0.5cm length), in a staged set up for photography that also provides an idea of their comparative sizes.

composed mainly by 2,3-dimethyl-1,4-benzoquinone (Hara et al. 2005).

The spider *Scytodes globula* is a nocturnal, sit-and-wait predator retreating to the underside of palm fronds inside rotting logs during the day. These spiders feed on a variety of arthropods, including other spiders and insects (Nentwig 1985; Li et al. 1999). *Scytodes globula* is slightly larger than the harvestman (Fig. 1).

Collection and maintenance in the laboratory.—We manually collected 106 adult male and female spiders and 114 harvestmen at the Parque Esporte Para Todos in the University of São Paulo, São Paulo City, State of São Paulo, southeastern Brazil (23°32′51″ S, 46°38′10″ W), from February to April (end of rainy season) 2009. We numbered the animals, maintained them in individual plastic boxes (12 cm × 8 cm × 4 cm height) and fed them on moistened dog food (for harvestmen) and crickets (for spiders) once a week. We used crickets both as a food souree for the spiders and as a control during the experiments. Because the spiders were collected as adults and therefore have probably eaten a wide variety of prey throughout their lives, we do not believe that the few crickets they have eaten in our laboratory influenced their behavior in the experiment. We provided water in a cotton ball for both species and maintained both an ambient temperature (25–30°C) and a natural light cycle (approximately 12:12 light: dark cycle). After the study, we fixed some animals in 70% ethanol and deposited them in the Museum of Zoology of the University of São Paulo, and released others at the same site where we had collected them.

Experiments.—We starved the spiders for 25–30 days before the trials to maximize their motivation to attack the prey (protocol previously tested in Souza & Willemart 2011). We used each animal only once. We used Sony Handycam DCR-TRV361 and DCR-HC65 NTSC, both with 'nightshot' (dim light), with no tripod to pick better angles for the movies. The arenas used in all experiments were 12 × 8 × 4 cm in height, with moist soil on the bottom.

Experiment 1, survival rate: To test if *S. globula* preys on *D. invalidus*, we randomly assigned 32 spiders to either harvestmen (8 males and 8 females) or 16 crickets used as a control (approximate body length equal to that of harvestmen – see Fig. 1). We left each pair for 5 d in the test arena and monitored the animals daily for prey capture between 12:00 and 13:00. We recorded the number of prey still alive on the fifth day, comparing the cricket and the harvestman groups. Because our interest was in testing the efficiency of spitting, we used a cotton ball to clean the arenas every day at the time we checked them for predation, specifically to remove the silk that some spiders had left.

Experiment 2, details of the interactions: Here we were interested in describing details of the behavior of *S. globula* (n = 20) against either *D. invalidus* (n = 7) or *Gryllus* sp. (n = 13). We randomly assigned spiders to one of the treatments. To reduce stress, we introduced the spider into the test arena 8 h before the trial. We ran the trials between 18:00–23:00 (nocturnal period). We introduced the harvestman in a vial as far as possible from the spider, allowed it to acclimate for 2 min, and then released it. We waited 10 min before declaring a trial ended with no interactions. We monitored and digitally recorded behaviors related to the approach between the two animals, and any physical interactions occurring for 10 s after all encounters, a period sufficient to detect whether the spider would start eating the prey after biting it. After the trial ended, spiders were returned to where they were being maintained, and uneaten prey were discarded. From the resulting videos, we created behavioral categories, quantifying and comparing their occurrences between treatments.

Experiment 3, testing the possible repellent effect of invisible secretions: The prior experiment and observations suggested that although we could not see anything, this species could be producing some kind of defensive barrier. We therefore tested the hypothesis that harvestmen regularly secrete small amounts of defensive secretions from the scent glands, invisible to the human eye. This would explain why *S. globula*

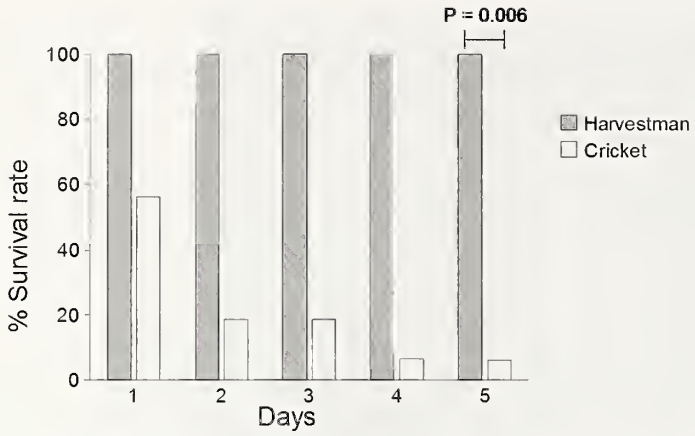

Figure 2.—Results of Experiment 1: survival rate of crickets (*Gryllus* sp.) and harvestmen *Discocyrtus invalidus* when paired with *Scytodes globula* for five days. The *P* value contrasts the number of prey that were still alive in the fifth day in the two groups.

would reject *D. invalidus*. We used four different treatments: harvestmen with glands experimentally obstructed with glue (n = 13), harvestmen with glue on the dorsum 3 mm from the gland (n = 13), crickets with glue on the dorsum (n = 14) and crickets with no glue (n = 14). We applied the glue 15 d before the trial to minimize the influence of residual odors. Because harvestmen secretions are extremely volatile (Gnaspini & Hara 2007), it is highly improbable that residual effluents were still on their body when we ran the trials. These were run between 18:00–23:00 (nocturnal period). All remaining procedures were the same as described for Experiment 2. From the videos, we quantified behavioral categories and compared their occurrences across treatments.

RESULTS

Experiment 1.—By the end of the fifth day, even after the spiders were severely starved and subsequently offered no other choice but harvestmen as a food choice, all the harvestmen were alive and more than 90% of the crickets were dead with their body contents emptied (X^2_1 = 7.69, P = 0.006) (Fig. 2). The spiders preyed upon 81% of the crickets during the first day of the experiment. We did not detect injuries among harvestmen, and all spiders were alive after the experiment.

Experiment 2.—Because experiments 2 and 3 involved recordings of interactions among spiders, harvestmen and crickets, and because the glue in experiment 3 did not affect the behavior of either prey or predator, we pooled observations of experiments 2 and 3 in this speeific analysis to better describe such interactions. Whenever possible, we employed behavioral categories previously used in the literature, such as those of Gilbert & Rayor (1985), Li et al (1999) and Souza & Willemart (2011). When spiders interacted with crickets (n = 41), the usual predatory sequence involved contact, spitting, and biting (Table 1, Fig. 3A). Biting was followed by "shake" in 40% of the observations, and all the trials ended with spiders eating the crickets. In contrast, spiders interacting with harvestmen (n = 33) seldom spat (n = 3) and never bit, generally touching the harvestmen and remaining still (Table 1, Fig. 3A). We did not observe emission of defensive secretions from the scent glands by the harvestmen or mechanical defenses such as pinching with chelicerae, pedipalps, or with the spines of legs IV (Fig. 3B).

Experiment 3.—As in experiments 1 and 2, data from Experiment 3 revealed a significant difference in the survival rate between harvestmen and crickets (X^2_1 = 21.03; P < 0.001) (Fig. 4). Obstructing the glands did not interfere with the survival rate of the harvestmen (Fisher exact test, P = 1). The glue per se had no influence on the results (crickets with glue vs. crickets without glue: Fisher exact test, P = 1).

DISCUSSION

Contrary to our expectation, *Scytodes globula* did not take advantage of its specialized spitting mechanism to prey upon the harvestman *D. invalidus*, even in starvation and with both sharing a small arena for five days. To understand why the spider rejected the prey, we conducted further detailed observations and experiments. Secondary defenses (defensive mechanisms used only after the predator is detected – Edmunds 1974) did not play a role. We observed no mechanical defense in Experiment 2 and, according to Experiment 3, rejection was not mediated by chemicals from the scent glands.

Chemicals from the scent glands have been extensively studied and identified as responsible for some predators rejecting harvestmen (Gnaspini & Hara 2007). *Discocyrtus invalidus* is known to release mainly 2,3-dimethyl-1,4-benzoquinone (Hara

Table 1.—Behavioral repertoire of the spider *Scytodes globula* when interacting with potential prey.

Category	Definition
Approach	To move towards prey
Bite	To lean forward and pinch prey with the chelicerae
Contact leg	Active or passive contact between a spider leg and prey
Dorso-ventral step	With the body off the substrate, to rapidly move the femur or tibia of legs II, III and IV up and down, as if it was walking but without displacement
Motionless	Not moving the body or the appendages
Retreat	To walk away from the prey after touching it
Pull	After extending legs I and placing tarsi I on the prey, to draw the prey in by flexing legs I
Shake	To move legs II, III and IV back and forth with short quick movements so that the body trembles, while touching the prey with legs I
Spit	To eject a sticky secretion from its chelicerae
Orient to prey	To rotate the body without displacement, ending with the anterior portion of the body facing the prey
Wave	To move legs I dorso-ventrally, slower than "Dorso-ventral step" and without contact with the substrate
Wrap	With alternated movements of legs IV, to take silk from the spinnerets and wrap the prey in silk

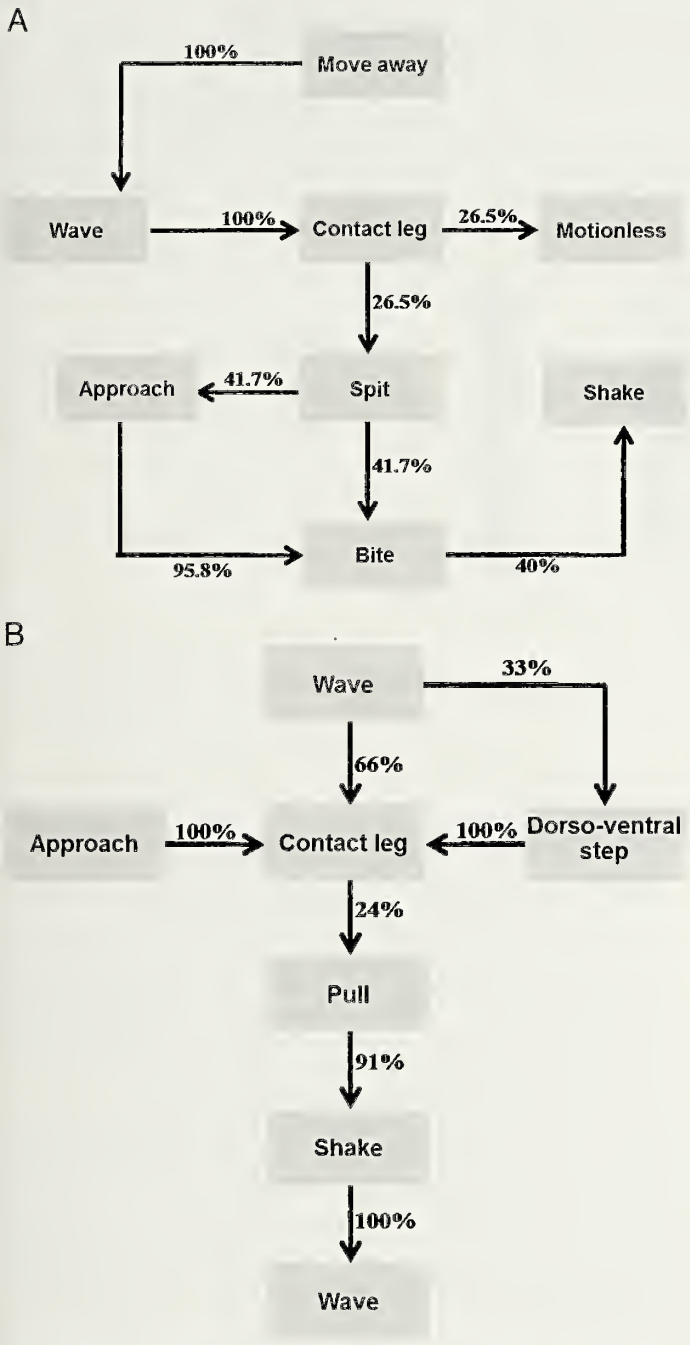

Figure 3.—Fluxograms of the predatory interaction between (A) the spider *Scytodes globula* and the cricket *Gryllus* sp. and (B) the spider and the harvestman *Discocyrtus invalidus* (see behavioral categories in Table 1). For the sake of clarity, only behavioral sequences with frequencies higher than 10% are included.

et al. 2005) by emitting a droplet that may run backwards in the grooves of the dorsal scutum (Hara & Gnaspini 2003), but this was never observed in our trials. Sabino & Gnaspini (1999), Eisner et al. (2004) and Willemart & Pellegatti-Franco (2006) also describe laniatorid harvestmen known to exude secretions when handled that failed to do so when attacked by spiders. Hara & Gnaspini (2003) triggered the exudation of secretions by holding *D. invalidus* with forceps, which is probably interpreted as more threatening than contact with the legs or even the glue spat by *Scytodes globula*.

Spiders have been reported to change their predatory strategies according to the type of prey (reviewed in Clements & Li 2005; Pekár & Lubin 2009). The same behavioral categories were used by *S. globula* with harvestmen and crickets, but with different frequencies. Though it does not appear in the fluxogram because they were very rare behaviors, "dorso-ventral step" and "pull" were observed twice each (distinct spiders and distinct harvestmen) against crickets. Similarly to Li et al. (1999) and Ades & Ramires (2002), crickets and harvestmen were often attacked or rejected after contact, so that behavioral categories like "contact leg," "pull," "wave," and "shake" probably inform the spider on the size/profitability/danger offered by the prey. Since our data suggest that chemicals from the scent gland secretions do not play a role in the harvestmen's response, spiders may be responding to the hard integument or, alternatively, repellent chemicals embedded in the cuticle. Whatever the explanation, a primary defense (defensive mechanisms present even in the absence of predators – cf. Edmunds 1974) could be playing a role, rather than the well-known chemicals from the scent glands.

Spiders spat on harvestmen in only three cases, and in those cases the harvestmen seemed unaffected except that they had to flee, dragging soil and pieces of dry leaves glued to their legs. Since capturing prey is costly, and spiders control the amount of venom they use according to the prey (Wigger et al. 2002; Wullschleger & Nentwig 2002; Nelson & Jackson 2011), an alternative hypothesis to explain the rejection is that the benefits provided by capturing a harvestman may not outweigh the cost of producing the amount of glue/venom necessary to subdue the harvestman. Moreover, a harvestman dragging leaves produces substrate-borne vibrations and air displacement, and the odor of the glue/venom may even act as a kairomone. These mechanical and chemical cues could potentially attract spider predators.

We have studied the predatory interactions between two harvestmen and spider species in our laboratory so far. The harvestmen *D. invalidus* and *Mischonyx cuspidatus* are avoided by the large ctenid *Enoploctenus cyclothorax* (Bertkau 1880) (Willemart & Pellegatti-Franco 2006; Souza & Willemart 2011) and the spitting spider *S. globula* (this study), both under starvation conditions. Factors such as being larger (*E. cyclothorax*) and having the ability to capture prey from a distance by spitting (which we had hypothesized would allow *S. globula* to avoid secretions and pick exact spots to bite) are therefore not indications that these spiders will feed on such harvestmen. If overcoming the thick cuticle were the issue, we would expect that even larger spiders, such as *Ctenus ornatus* (Keyserling 1877), would eat the harvestmen if they are able to pierce the prey's integument. We would also expect that spiders with weak chelicerae that build sheet webs and actually prey upon these harvestmen (such as the recluse spider *Loxosceles* sp.), would take advantage of an immobilized prey to pick specific vulnerable spots (such as articulations) to bite the prey. We are currently studying these spiders in our laboratory. Such studies, in addition to this and previous papers, may add to the understanding of proximate causation of prey acceptance and rejection in spiders.

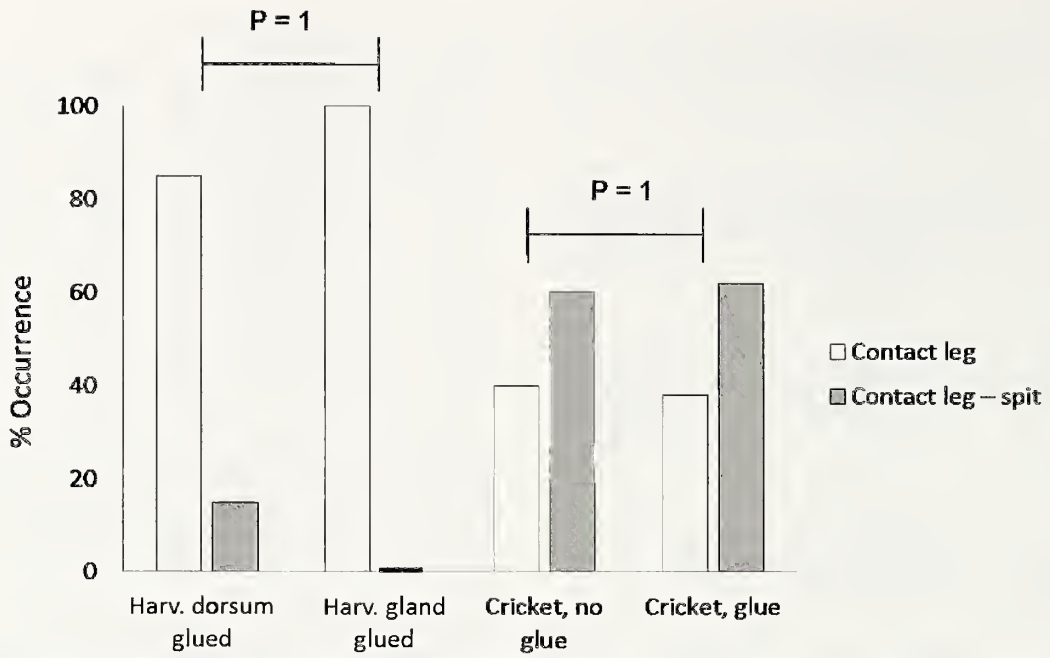

Figure 4.—Behavior of *Scytodes globula* when interacting with crickets with or without glue on the dorsum (control groups) and harvestman *Discocyrtus invalidus* with glue on the dorsum (control group) and glue clogging the scent gland opening (treatment group). Harv. = harvestman.

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Static allometry and sexual size dimorphism in *Centruroides margaritatus* (Scorpiones: Buthidae)

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Abstract. Animal body traits are scaled relative to overall body size depending on the evolutionary context. Most naturally selected traits are scaled approximately isometrically (constitute a constant proportion of the body size at different body sizes), whereas those under sexual selection tend to present positive static allometry (be proportionally larger in larger individuals). However, there are body traits that might be influenced by both natural and sexual selection. We studied the courtship behavior of the scorpion *Centruroides margaritatus* (Gervais 1841) and analyzed the static allometry of several body traits. We hypothesized that those traits that were actively used in courtship and seemed to be sexually dimorphic could be under sexual selection. The main sexually dimorphic traits were body size (female larger) and metasoma length (male longer). Although metasoma length of males had a steeper allometric slope (larger males had longer metasoma) than that of females, the slopes did not differ significantly. All body traits measured showed isometry with body size, except that the pecten presented negative allometry in males. Thus the length of the metasoma of males, thought to be influenced by sexual rather than natural selection, did not present positive allometry as expected. Males used the metasoma actively while courting females.

Keywords: Courtship behavior, isometry, sexual selection, natural selection

Variation in the size and shape of particular body parts relative to variation in body size at a given life stage (static allometry) is generally thought to be the result of either sexual selection or natural selection (Darwin 1879; Andersson 1994; Emlen 2008). If a male morphological feature has evolved as a weapon in fights with other males, a positive allometric relationship is predicted (Huxley 1932; Schroeder & Huber 2001; Kodric-Brown et al. 2006) (but see Eberhard 2002; Bondurianski & Day 2003). In this context, larger males would have proportionally larger weapons than smaller males ($b > 1$ in a log-log regression of size of a given structure on body size). This is because individuals with larger weapons usually have an advantage in resolving direct male-male battles (Eberhard & García-C 1999; Eberhard et al. 2000; Schroeder & Huber 2001). Thus, positive allometry should be expected in secondary sexual characters under strong intrasexual selection. However, male genitalia, which are thought to evolve under intersexual sexual selection (Eberhard 1985, 1996), nearly always have negative allometry ($b < 1$) (Eberhard 2008). This may be expected if it is advantageous for males to have "standard sized genitalia" that would fit the most common size of females in the population according to the 'one-size-fits-all' hypothesis (Eberhard et al. 1998). Alternatively, such a standard size in genitalia may be more efficient to transfer sperm: a natural selection role (House & Simmons 2003).

The relative size of traits in which both sexual and natural selection forces are involved is less predictable. Traits under the exclusive influence of natural selection are expected, at least in most cases, to have isometric relationships with body size (b equal to or nearly equal to 1, Eberhard et al. 2009), though this is not always the case (see Klingenberg & Zimmermann 1992). It is common in a wide range of animal species that legs, antennae, wings, horns, and other structures are used in the context of both natural and sexual selection (Eberhard 1996, 2004, 2010). For instance, in a large number of spider species adult males not only use their legs (particularly leg I) and chelicerae to court their mates (Stratton et al. 1996; Eberhard & Huber 1998; Barrantes 2008; Aisenberg & Barrantes 2011), but also as walking and killing and feeding organs, respectively. Hence, given the dual or multiple functions of a trait subject to the combined action of natural and sexual selection, the type of allometric relationship is difficult to predict. In these cases, a more reliable approach to evaluate the effect of sexual and natural selection on particular body parts relative to body size is to compare the relative magnitude of the slopes across traits between sexes (Eberhard et al. 2009). In addition, this approach allows using different body features as controls for one another (Rodríguez & Al-Wathiqui 2012).

The courtship behavior of scorpions includes a series of tactile and vibratory stimuli produced with parts of the male's body that are used in both sexual and non-sexual contexts (Polis & Sissom 1990; Lourenço 2000). Typically, the courtship in scorpions is roughly divided into four phases: initiation, promenade a deux, sperm transfer and termination (Polis & Farley 1979; Tallarovic et al. 2000). Detailed descriptions of all these phases for different species can be found in Polis and Sissom (1990) and Lourenço (2000). Some male behaviors occur in more than one phase, while others are restricted to only one. For instance, shaking and rocking the body back and forth while standing immobile (juddering), and spreading out the pectines, often sweeping the substrate with them, occur in most phases. In contrast, the male contacting the female with his metasoma and sting ("sexual sting") and using the metasoma to club the female occur only during the initiation phase (Polis and Sissom 1990). The male grasping of the chelae of the female with his own to guide her during the dance and cheliceral massages occurs during both the promenade a deux and sperm transfer. The use of multiple body parts in the courtship behavior (and in other non-sexual functions) of scorpions is convenient for examining how the size of these body parts change in relation to change in body size.

Our study has a twofold objective: to complement the description of the courtship behavior of *Centruroides margaritatus*

(Gervais 1841) (family Buthidae) provided by Briceño and Bonilla (2009), and to analyze the sexual dimorphism and allometric relationships of those body traits involved in the courtship behavior of *C. margaritatus*. In addition we include other traits that are not directly involved in the courtship behavior (e.g., leg IV) as well as female morphological traits in the allometric analysis for comparative purposes. We expect a positive allometric relationship (or at least a steeper slope) for the length of male metasoma since this structure is longer in adult males than in females, despite the smaller size of males, and it is used actively during courtship. Considering a possible effect of sexual selection, we also expect a steeper slope for male traits relative to the same female traits and for male traits involved in courtship relative to those that are not.

METHODS

Courtship behavior.—We video-recorded the courtship behavior of one adult pair of *C. margaritatus* (collected by E. Arévalo, at Atenas, Alajuela Province; 9°58′N, 84°26′W; 1013 m elevation) to obtain a detailed description of the different behaviors involved in courtship as a baseline to select the morphological traits to measure. The female and male were housed individually in plastic containers: 24.3 cm length, 14.3 cm width, 6.8 cm height for the female's container and 13.7 cm, 12.8 cm, 5 cm for the male's container. We covered the bottom of each container with approximately 2 cm of sand and placed a small rock near a corner of the female's container to provide a suitable surface on which the male could deposit the spermatophore. During captivity scorpions were offered crickets (*Acheta domesticus*) twice a week and water ad libitum.

Material examined.—We measured the area, width and length of the carapace, length and width of the chela, segments of the metasoma, telson, pecten, and patella of legs I and IV of 25 adult males and 19 adult females in the Arachnological Collection of the Museo de Zoología, Universidad de Costa Rica that were collected in different localities of the Central Valley and northwestern region of Costa Rica: UCR 7 (1 ♀), UCR 8 (1 ♀), UCR 20 (1 ♀), UCR 21 (1 ♀), UCR 24 (1 ♀), UCR 31 (1 ♀), UCR 33 (1 ♀, 2 ♂), UCR 36 (1 ♀), UCR 49 (1♂), UCR 53 (1 ♀), UCR 66 (1 ♂), UCR 86 (1 ♂), UCR 163 (1 ♂), UCR 164 (1 ♀), UCR 183 (1 ♀), UCR 187 (1 ♂), UCR 188 (1 ♀), UCR 189 (1 ♂), UCR 190 (1♀, 1 ♂), UCR 193 (1 ♂), UCR 195 (1 ♀), UCR 197 (1 ♀), UCR 201 (1 ♂), UCR 211 (3 ♂), UCR 212 (1 ♀, 3 ♂), UCR 213 (1 ♂) ,UCR 215 (2 ♂), UCR 214 (1 ♀), UCR 218 (1 ♀), UCR 220 (1 ♀, 2 ♂), UCR 223 (3 ♂). We photographed each body part under a dissecting microseope using a Nikon Coolpix 4500 camera and then measured the different body parts using the program UTHSCSA Image Tool v. 2.1.

Statistical analyses.—We examined the effect of sex on size variability of several different body parts using a Multivariate Analysis of Variance (MANOVA). We then correlated the square root of the area, width and length of the carapace (males: $r = 0.99$, $P < 0.000001$, same value for area vs. width and length of carapace males; females: $r = 0.91$, $P < 0.00001$, and $r = 0.92$, $P < 0.00001$ for width and length of female's carapace respectively), and selected the carapace area as a measure of body size as all three variables are highly correlated. Although width and length of the carapace are frequently used as a measure of body size, its area is less prone to variation in length or width of the carapace (Gonzaga & Vasconcellos-Neto 2001). Next, we determined the relationship between change in body size (carapace area) and the size of each body part using regression analyses and analyses of covariance (ANCOVA) to compare slopes of each variable between both sexes. Then we compared the slopes, b, obtained from regressing the carapace area against each of the other variables between sexes, using a paired *t*-test. Though slopes calculated for each variable are independent between sexes, we used a paired *t*-test rather than a *t*-test for independent samples because this approach allows us to evaluate if in general the morphological features of a particular sex are larger (in proportion to the body size) than in the other sex. We chose ordinary least squares regression (OLS) over reduced major axis regression (RMA) for the following reasons: OLS describes the functional relationship (cause-effect) between variables; it distinguishes the effect of one variable on the other from the individual variation; it is very robust to variation of the error in x and does not underestimate slope values as had previously been argued (Al-Wathiqui & Rodríguez 2011). We used the R Statistical Language (version 2.14: http://cran.r-project.org) for all statistical analyses and $\log_{10}$ transformed all variables prior to regression analyses.

RESULTS

Courtship behavior.—A few seconds after the male had been placed in the female cage, she walked a few steps toward the male. As soon as the male detected her (possibly by substrate vibrations), he started to judder, rocking his abdomen rapidly dorso-ventrally. At the same time he spread his pectines outward and downward, moving them forward and backward, sweeping the ground. The female walked toward the male, coiling her metasoma over her midline. She often moved her metasoma down and to one side for a few seconds, raising it again as she approached the male. The male also moved forward toward her, juddering nearly continually. Once he got close, he seized the patella of her left pedipalp with his right pedipalp, and then moved his left pedipalp and grasped her right chela. At the same time the female maintained her metasoma coiled and tilted toward one side. Often she slightly uncoiled its basal segments, extending them toward the male, but she kept the fifth segment and the telson coiled. She also swept the ground with her pectines, but less frequently than the male.

Once the male had grasped the female's chelae, he began to move forward, pushing the female backward. He then switched and began to walk rapidly backward, pulling the female toward him, holding her chelae. He continued to sweep the ground with his pectines. This stage is described as the promenade a deux (Polis & Farley 1979). On several occasions the female briefly resisted the male's pulls; in response he juddered and pulled her toward him by the chelae. If the female still resisted, the male juddered again, apparently with more intensity, and extended his first legs, contacting the base of female's pectines and rubbing them with a more or less circular movement of his tarsi.

Several times during the promenade a deux phase the pair walked over a rock, and sometimes the female stopped on top of it. The male beat the rock surface rapidly with alternate

Table 1.—Mean (mm) and standard deviation (SD) of morphological variables for males and females and statistical (ANOVA) comparisons.

Variable	Mean male	SD male	Mean female	SD female	$F_{(1/42)}$	P
Carapace area	7.13	1.18	7.94	1.28	4.60	0.0378
Carapace length	7.94	1.33	8.83	1.33	4.65	0.0369
Carapace width	7.58	1.20	9.07	1.44	13.73	0.0006
Chela width	3.08	0.54	3.66	0.82	6.98	0.0115
Pincer length	7.94	1.33	8.83	1.40	0.98	0.3272
Metasoma seg. 1	8.51	1.85	6.86	1.29	9.57	0.0035
Metasoma seg. 2	10.49	2.35	8.47	1.42	9.33	0.0039
Metasoma seg. 3	11.48	2.61	9.09	2.61	10.91	0.0019
Metasoma seg. 4	11.82	2.49	9.65	1.72	9.24	0.0041
Metasoma seg. 5	12.09	2.41	10.18	1.83	7.19	0.0104
Metasoma length	54.39	11.56	44.25	7.85	9.35	0.0039
Telson length	6.57	1.32	5.96	0.98	2.34	0.1333
Pecten length	7.70	1.24	7.38	1.30	0.73	0.3961
Patella leg I	4.40	0.72	4.67	0.72	1.18	0.2842
Patella leg IV	7.24	1.41	7.48	1.48	0.29	0.5949

movements of his first legs (drumming, hereafter), and the female responded to this behavior by lifting her abdomen dorsally, allowing the male to rub her pectines as described above.

The promenade a deux is thought to allow the male to search for a suitable surface on which to deposit his spermatophore (Polis & Farley 1979). The male pulled the female to the rock more than eight times; the last time she stayed still for several seeonds almost in the middle of the rock. The male immediately began to move his pedipalps alternately up and down, while grabbing female's chelae with his own chelae. The movements of his pedipalps became faster and simultaneous rather than alternate, as he deposited his spermatophore. Immediately afterward, the female moved forward and the male started drumming the rock with his first legs. The female continued moving forward, while the male grabbed her chelae.

The male then drummed, uncurled and extended his metasoma forward (leaving the telson curled), reaching the anterior, dorsal section of the female metasoma. It appeared as if he were trying to strike her with the posterior end of his metasoma. During this struggle she freed herself from his pedipalps, turned her body ca. 180°, and clubbed the male with the posterior end of her metasoma while he was juddering. Then she walked about 10 cm from the male. The male approached her but she clubbed him twiee with her metasoma and he moved away.

The video-reeordings showed that the up-and-down fast movements of the male pedipalps occurred while the male was extruding his spermatophore. It was also evident from the video that a thread-like structure eonnected the spermatophore to the male opercula after it was attached to the rock, and that the spermatophore changed from a nearly horizontal position to a vertical position when the male walked toward the female. At the end the female moved away without accepting the spermatophore.

Morphometric analysis.—A significant amount of the combined variance of all morphological variables was explained by differences between sexes (MANOVA: Pillai test = 0.89, df = 15/28, $P < 0.000001$). All three measures of the carapace (area, length, and width) were significantly larger in females than in males (Table 1). The width but not the length of the chelae was greater in females than males (Table 1). Each segment of the metasoma, and consequently the total length of the metasoma, was longer in males than females (Table 1). The lengths of the telson, pecten and patellae of legs I and IV did not differ significantly between males and females (Table 1).

All morphological variables of males and females increased with body size (carapace area) (Fig. 1, Table 2A). Metasoma segments, particularly segments 1 and 2, and total metasoma length increased faster with body size in males than in females, but slopes did not differ significantly between sexes for any of these variables, based on pairwise comparisons (Table 2A). The width and length of the chelae, as well as the other body parts (the lengths of the telson and of leg patella I and IV) also showed similar increments with body size for both males and females (Table 2A, Fig. 1). However, slopes calculated for morphological features of males were overall significantly larger than those calculated for the same features in females (paired t-test: 3.21, df = 10, P = 0.008), indicating that in relation to body size, body parts of males increased faster (except for pecten length and chela width, Table 2A). All body parts scaled isometrically (b = 1) on body size, except for the length of the pecten in males. Peeten length in males differed in having a negative allometry (Table 2A, Fig. 1). The slope for patella IV vs. patella II showed an isometric relationship for females, but a positive allometry for males (Table 2B).

DISCUSSION

The courtship and sperm transfer of *C. margaritatus* is very stereotyped. With the exception of two behaviors, the contact of the anterior section of the female pectines with the tarsus of male first legs and the movement of the male pedipalps during the spermatophore extruding, the courtship of *C. margaritatus* is very similar to the courtship of many other species (Polis & Sissom 1990; Lourenço 2000). We documented the contact of female pectines in two other species of *Centruroides* (*C. bicolor* Pocock 1898, *C. limbatus* Pocock 1898: C. Sánehez-Quiroz unpubl. data), and Lourenço (2000) described the same behavior in *Tityus fasciolatus* (Pessa 1935). We also documented the movement of the male pedipalp during spermatophore extrusion in these two other *Centruroides* species (C. Sánchez-Quiroz unpubl. data), suggesting that both of these

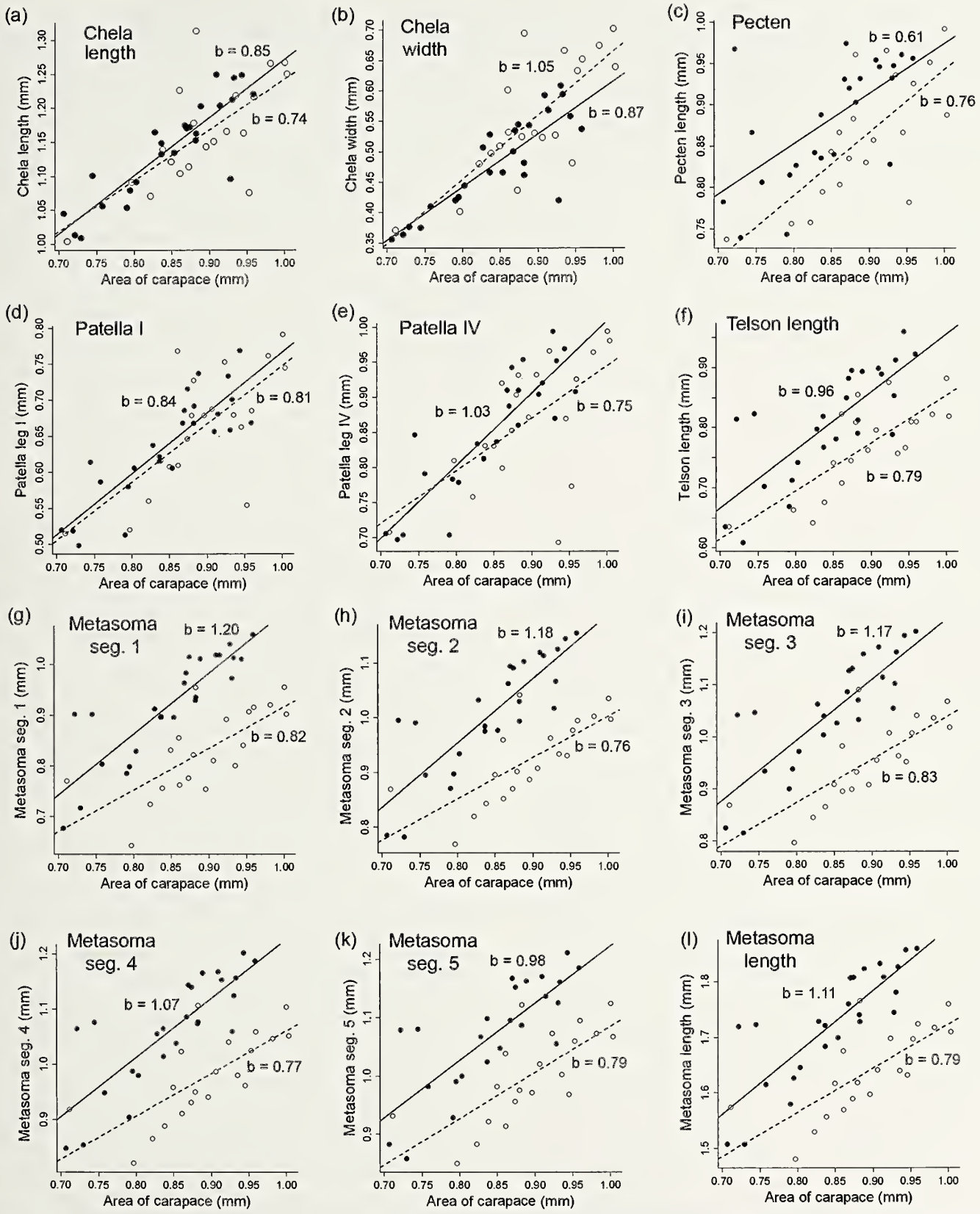

Figure 1.—Relationship of the square root of the carapace area to the other 12 morphological traits. The continuous line and black circles correspond to the area of the carapace of males regressed against each of the other morphological traits, and the dashed line and empty circles correspond to the area of the carapace of female regressed against each of the other morphological traits. Slope values for males and females are included. All variables were $\log_{10}$-transformed.

Table 2.—Allometric relationship between different morphological variables, with the square root of carapace area as a measure of body size. It includes *F*-test for the slope (H_0: b = 0), the slope value, the proportion of the variance of each dependent variable explained by carapace area (r^2) for males and females and a $P_{(bm\text{-}bf)}$ value of the comparison between male-female slopes. All variables were $\log_{10}$-transformed. Negative allometry is indicated by *, and positive allometry by **.

Variable	Males				Females				$P_{(bm\text{-}bf)}$
	$F_{(1/23)}$	b	r^2	P	$F_{(1/18)}$	b	r^2	P	
A, carapace area (independent variable)									
Chela width	57.39	0.87	0.71	< 0.00001	27.31	1.05	0.60	0.00006	0.41
Chela length	80.83	0.85	0.78	< 0.00001	16.36	0.74	0.48	0.00076	0.58
Metasoma seg. 1	70.45	1.20	0.75	< 0.00001	18.54	0.82	0.51	0.00042	0.11
Metasoma seg. 2	55.07	1.18	0.70	< 0.00001	21.87	0.76	0.55	0.00019	0.07
Metasoma seg. 3	48.09	1.17	0.68	< 0.00001	24.89	0.83	0.58	0.00009	0.16
Metasoma seg. 4	43.47	1.07	0.65	< 0.00001	19.06	0.77	0.51	0.00037	0.22
Metasoma seg. 5	37.13	0.98	0.62	< 0.00001	20.34	0.79	0.53	0.00027	0.44
Metasoma length	51.81	1.11	0.69	< 0.00001	21.58	0.79	0.54	0.00020	0.17
Telson length	36.01	0.96	0.61	< 0.00001	27.38	0.79	0.60	0.00006	0.45
Pecten length	14.61	0.61*	0.40	0.0009	19.91	0.76	0.52	0.00030	0.51
Patella leg I	63.66	0.84	0.73	< 0.00001	17.88	0.81	0.50	0.00050	0.86
Patella leg IV	71.26	1.03	0.76	< 0.00001	10.58	0.75	0.37	0.00441	0.25
B, patella IV (independent variable)									
Patella leg I	399.6	1.17**	0.95	< 0.00001	36.26	0.88	0.67	< 0.0001	0.07

behaviors are also widespread among the New World Buthidae.

Sexual size dimorphism is notable between females and males of *C. margaritatus*. Body size of females (considering the area of the carapace as an index of body size) is larger than the body size of males. This is a general pattern in scorpions (Polis & Sissom 1990) and is an expected consequence of the different reproductive role played by each sex (Williams 1966; Prenter et al. 1998). In arthropods, the reproductive success of females is directly related to body size, and larger females are capable of carrying either larger number of eggs (or embryos) or larger eggs (or embryos) (Andersson 1994), though evolution of sexual size dimorphism has possibly been influenced by many different factors rather than only by differences in reproductive role (Hormiga et al. 2000; Barrantes 2008). Females of *C. margaritatus* also have more robust (wider) chelae. The size of the chelae varies in scorpions between sexes and across genera (Meijden et al. 2009). In *Centruroides* and other genera (e.g., *Heterometrus*, *Isometrus*), females have more robust chelae, but the opposite pattern is found, for example, in *Buthus* and *Scorpio* (Polis & Sissom 1990). Sexual differences in the dimensions of the chelae are expected to be related to different diet and size of prey captured or differences in the use of chelae in courtship behavior. However, there is not enough information on scorpions to separate the effects of diet and courtship on the morphological design of the chelae (Polis 1979; McCormick & Polis 1990; Benton 1992).

Males of *C. margaritatus* are smaller than females but have longer metasomas, and in general body parts increase faster (relative to body size) than in females. Presumably, male *C. margaritatus* reach adulthood one molt earlier than females, as occurs in *C. gracilis* (Latreille 1804) (Franke & Jones 1982), and the smaller size in males is thus a direct consequence of their early maturation. An early maturation may provide males with the advantage of an early breeding start relative to females and a greater maneuverability that may allow them to decrease predation risk (Andersson 1994). On the other hand, the difference in the length of the metasoma is manifested only in adult scorpions (Polis & Sissom 1990; Lourenço 2000); in previous stages length of metasoma is similar in both males and females. This seems to be a general pattern in scorpions, though no data are available for *C. margaritatus*. It is likely that the longer metasoma in males is the result of a faster growth rate (acceleration: Reilly et al. 1997). In contrast, the size of females may increase more gradually, since they have one more molt (hypermorphosis, Reilly et al. 1997). Since males have a longer metasoma, and this feature is present only during adulthood and the metasoma is used in male-female sexual interactions, it is expected that the longer metasoma in adult males is related to its sexual role.

With the exception of the length of the pecten, which had a negative allometric relationship, all other morphological features of *C. margaritatus* had a proportional change relative to body size (b = 1). It is often stated that traits evolved under sexual selection have a positive allometry (b > 1) relative to body size, based on the assumption that larger individuals benefit more in allocating more resources to the growth of the selected trait than small individuals (Huxley 1932; Gould 1974; Green 1992; Kodric-Brown et al. 2006). However, the cost and benefit of producing and carrying proportionally larger structures likely varies both among different traits and among different species (Eberhard 2002; Bonduriansky & Day 2003). The balance between cost and benefit in producing and carrying sexually selected traits, and the counterbalancing effect of sexual selection and natural selection acting on the same trait, may yield relationships between the size of a trait and body size that differ from positive allometry, converting the absolute value of 1.0 to an unreliable indicator of the existence or absence of sexual selection (Bonduriansky & Day 2003). In fact, positive allometry seems to be the exception and not the rule for traits used by males as weapons and signaling devices that evolved through sexual selection (Bonduriansky & Day 2003; Bonduriansky 2007). Thus, the isometry of

different traits in *Centruroides* that are thought to have evolved under sexual selection follows the more general trend (even scaling of a trait on body size) found in many other species (Eberhard 2002; Bonduriansky & Day 2003; Bonduriansky 2007). The positive allometry of patella I relative to patella IV in males, but their isometric relation in females, supports the argument that body structures under sexual selection can scale differently on body size. In this case both patellae are homologous, but the patella IV is used as a control (Eberhard et al. 2009; Rodriguez & Al-Wathiqui 2012), since sexual seleetion is expected to affect patella I but not patella IV (see description of courtship behavior).

The even scaling of nearly all traits on body size in *C. margaritatus* may be related to the function of such body parts and the counteracting effect of sexual and natural selection on the same traits. It is possible that body parts used as tactile signaling devices (e.g., tarsus I, chelae) have a more or less "standard size" that is appropriate for stimulating most females in the population (mean female size) (W. Eberhard unpubl. information). In many other arthropods, selection favoring standard non-genital contact structures (e.g., genital clasping structures) tends to result in isometry or even negative allometry (Eberhard 1996, 2004, 2010). Thus, each of the structures used by male *C. margaritatus* to stimulate females in a specific area may be also under stabilizing selection, similar to the genital clasping structures. A similar scaling pattern between body parts and body size in *C. margaritatus* may result from the balanced effects of natural and sexual selection acting on the same trait (Bonduriansky & Day 2003). For instance, if longer first legs are more suitable for stimulating females but have a negative effect on survival, the balanced effect of both sexual and natural selection may result in an even scaling of leg I on body size. Hence, the interaction of the natural and sexual selection (Elgar & Fahey 1996), as well as the selection for standard size of non-genital contact structures (Eberhard 2004, 2010), likely affects the shape and design of some body parts in male *C. margaritatus*, acting as stabilizing forces that result in a proportional size of these body parts relative to the body size.

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SHORT COMMUNICATION

Nephila female gigantism attained through post-maturity molting

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Abstract. *Nephila* are known for the greatest degrees of sexual size dimorphism among orb weaving spiders (Araneoidea) and thus among terrestrial animals. However, a meaningful quantification of the dimorphism is lacking and the proximate developmental mechanisms of female gigantism are poorly understood, being attributed solely to female delayed maturation. Here we show that females in the giant wood spider *Nephila pilipes* (Fabricius 1793) become giants through facultative post-maturity molting, a phenomenon resulting in female carapaces on average 4.27 times longer than males' (ranging from 3 to 6.4 times), and female mass averaging 125 times the male's (ranging from 28 to 502 times). Although the small males follow a typical developmental pathway and reach maturity with their final molt, the females mature at varying sizes and instars and then continue to grow by molting the entire exoskeleton except their genitals. The newly discovered phenomenon of additional, single-sex, adult, non-genital molting may represent a critical developmental adaptation that facilitates female gigantism in *Nephila* as a response to fecundity selection.

Keywords: Sexual size dimorphism, development, fecundity selection, spider, *Nephila pilipes*

Sexual size dimorphism (SSD) describes a morphological syndrome in which male and female sizes differ significantly within a species. Although well-known vertebrate cases exist where males are the larger sex, more dramatic sexual size differenees are usually found in animals with female-biased SSD (Norman et al. 2002). A variety of selection pressures may account for the evolution of female-biased SSD; a commonly invoked explanation is fecundity selection, which postulates that increased female size enables greater egg production (Higgins 2002; Kuntner & Coddington 2009). However, in arthropods, evolution towards gigantic females acts against substantial developmental constraints, as arthropods can only grow by molting, which almost invariably ceases upon maturity (e.g., Stillwell & Davidowitz 2010; Foelix 2011).

Among terrestrial animals, the most extreme female gigantism is found in orb weaving spiders, clade Araneoidea (Foellmer & Moya-Laraño 2007). At least four independent phylogenetic origins of SSD are postulated, and these are predominantly cases of females becoming evolutionary giants (Hormiga et al. 2000). Classical cases of female gigantism have been reported in the family Nephilidae (Kuntner et al. 2008; Kuntner & Coddington 2009), in particular in the genus *Nephila* (Fig. 1a), although precise quantifications of sexual mass differences are mostly lacking. In addition, the proximate mechanisms of the developmental pathways responsible for extreme female gigantism are not well understood and mostly attributed to delayed maturation in females as a response to fecundity selection (Higgins & Goodnight 2010; Higgins et al. 2011). As most reports of SSD in spiders derive from scattered measurements of body length, and as body size is confounded with condition (Foellmer & Moya-Laraño 2007), body mass should instead serve as a better measure of SSD. Here, we report the range of female to male mass ratio as an accurate quantification of female gigantism and compare it to the carapace size ratio. In addition we report our discovery of a previously unknown developmental mechanism that underlies female gigantism in the giant wood spider *Nephila pilipes* (Fabricius 1793) (Fig. 1a).

We collected 155 adult males and 108 subadult females of *N. pilipes* on Pulau Ubin, Singapore (1.421575°N, 103.932542°E). In the laboratory, we reared the males in plastic cups and the subadult and adult females in their own webs made in frames. We fed the males fruit flies, and the females flies and mealworms thrice a week, and watered them daily.

In most spiders molting ceases with maturity, and this has also been conventionally presumed in *Nephila* and other araneoid spiders (for exceptions, see below). However, in our pool of 40 subadult females that were subjected to daily monitoring until their maturity, 27 (67.5%) molted even after maturity (mean number of molts = 1.15, range = 1–2; SD = 0.36, n = 27). Our morphological examination of 17 molts (or exuvia) that were shed after maturity revealed no molted genital structures. Each exuvium contained a hole in the part of the ventral abdomen that contains the genitals (Fig. 1b). No adult female molted after egg sac and plug formation (Kuntner et al. 2012).

Adult female carapace length ranged between 7.09 and 11.52 mm (mean = 8.80 mm, SD = 0.90 mm, n = 31), and that of males ranged between 1.80 and 2.36 mm (mean = 2.06 mm, SD = 0.15 mm, n = 50). Adult females weighed between 0.385 and 1.757 g (mean = 0.930, SD = 0.375, n = 31), and adult males between 0.0035 and 0.0137 g (mean = 0.0074, SD = 0.002, n = 98). In our population, females were on average 4.27 times larger than males in carapace length (ranging from 3 to 6.4), but were 125 times heavier than the males (with extremes ranging from 28 to 502 times male mass). According to the review of Foellmer & Moya-Laraño (2007), our SSD quantification in *N. pilipes* reflects the most extreme mass dimorphism reported in a terrestrial animal (for an extreme among marine animals, however, see Norman et al. 2002).

Post-maturity molting was more likely in females that copulated for a shorter total duration (Mann-Whitney U = 81.5, n = 39, P = 0.023), and, albeit non-significant, such a trend was also present in females exposed to lower levels of polyandry and fewer copulatory insertions (Mann-Whitney U = 100, n = 39, P = 0.088; number of males: χ^2 = 7.12, df = 3, P = 0.068). This suggests that females, regardless of their size and mass, may terminate their growth after possessing enough sperm for oviposition, but that they may continue to grow and molt if the number or duration of matings has been below a certain threshold. It is important to note that molting females

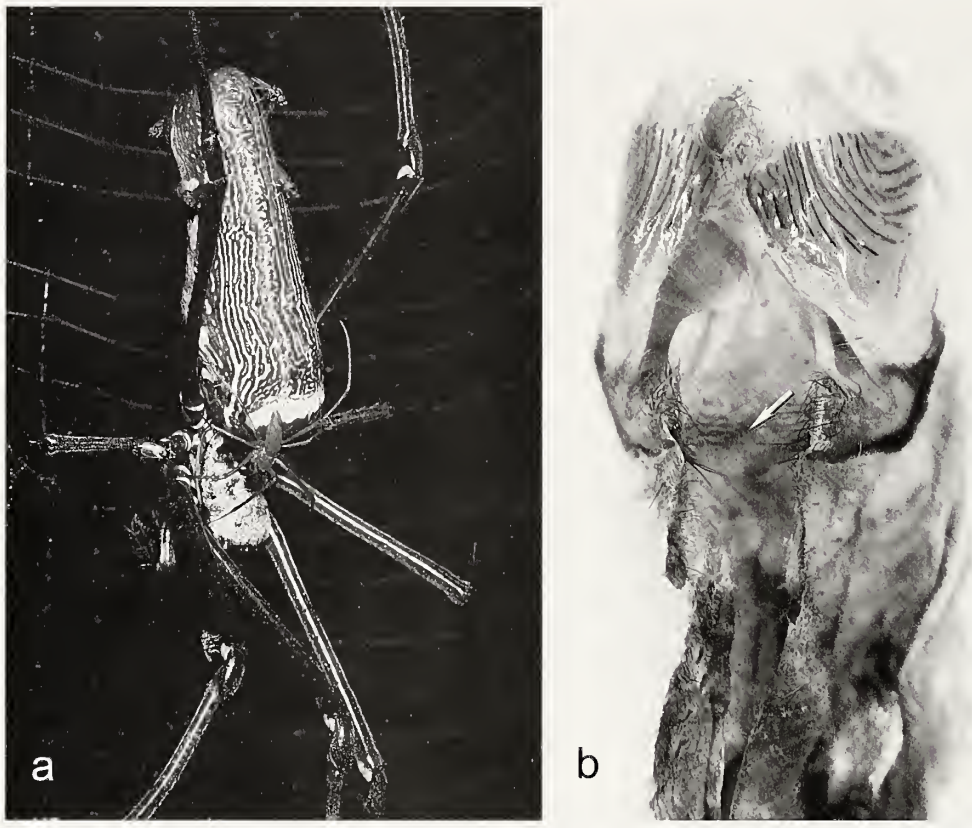

Figure 1a, b.—The giant wood spider *Nephila pilipes*, a highly polygamous and sexually dimorphic species whose females are on average 125 times heavier than males. a. A gravid and fully grown female in nature supporting on her body a conspecific male and six kleptoparasitic flies. b. Female adult molt containing the entire abdominal exoskeleton except the genital area (arrow).

do not shed their genitals and thus may retain sperm already stored. Such developmental plasticity may be a critical response to fecundity selection, which in *Nephila* results in extreme female gigantism. As reported here, females are freed from developmental constraints to be capable of growth beyond maturity and presumably increased egg production. Other explanations are possible, however. For example, chemical signaling by a molted female may attract more males, which may serve the female as she strives for polyandry (Kuntner et al. 2012). In support of this may be the case of *Nephila clavata*, where males are highly attracted to recently molted females (Miyashita & Hayashi 1996).

Our discovery of post-maturity female growth in *Nephila pilipes* is at odds with the conventional wisdom that most spiders exhibit determinant growth and do not molt after maturity (Foelix 2011). Post-maturity molting has predominantly been known in phylogenetically more ancestral and sexually monomorphic spiders such as liphistiids (Haupt 2003; Foelix 2011) and mygalomorphs (Baerg 1958; Miyashita 1992; Schmidt 1993); in these groups of long lived spiders, adult female molting involves growth by shedding the entire exoskeleton, including the genitals (Schmidt 1993; Foelix 2011). However, studies have also outlined cases of post-maturity molting in phylogenetically scattered examples of araneomorph spiders, such as social eresids, genus *Stegodyphus* (Kraus and Kraus 1988), female filistatids (Vetter 2011) and male *Loxosceles*, where, apparently, eunuch males engage in fatal post-maturity molting attempts (Vetter 2011). There exist some other reports for araneomorphs, but most are exceptional or anomalous (Fujii 2001; see also Kayashima 1981). For example, as the only known case of post-maturity molting in araneoids, Kaston (1968) reports it happened in five out of hundreds of black widow females (*Latrodectus mactans* and *L. hesperus*), and those molts, as in *Nephila*, did not contain any epigynal structures; molted females in fact retained enough sperm in their spermathecae to lay fertile eggs post molting. However, the rarity of post-maturity molting in *Latrodectus*, albeit suggestive of a potentially more widespread ability of araneomorph spider adults to molt, nevertheless suggests that it is not an obligate, but rather an anomalous life history trait (Kaston 1968).

Because other araneoid spiders do not usually molt as adults, the mechanism of non-genital molting of adult *Nephila* females may represent a critical developmental adaptation facilitating gigantism, and involves the additional dimension of sexual dimorphism. Although *N. pilipes* males follow typical spider developmental pathways and reach maturity with their final molt, females mature at varying sizes and then continue to grow by molting the entire exoskeleton except their genitals. However, it remains to be established how typical such development is for *Nephila*, or even if it is prevalent in all populations of *N. pilipes*, a widespread species ranging throughout South, Southeast and East Asia into Australasia (Su et al. 2007), and thus from rain forests to dry subtropical forests. Post-maturity molting was apparently unknown to Harvey et al. (2007), who revised Australasian *Nephila*. In their detailed studies of the Papuan population of *N. pilipes* (as *N. maculata*), Robinson & Robinson (1973, 1976) postulated 14 instars in the female and 7 in the male. They acknowledged some confusion as to the number of instars, but nevertheless only considered the last, 14th instar, to represent an adult female. Similarly, in a developmental study, Higgins (2002) did not report that several large instars in females from Papua might represent adult, not subadult females, although she concluded that males only go through four and females through about 10 instars. In our prior mating study on the Singapore population, we also failed to take this into account (Kuntner et al. 2009). We believe that instars 12 to 14 in the study of Robinson & Robinson (1976) represented adult females of different sizes, and that such plastic development will be typical of all populations of *N. pilipes*. In fact, we predict it also to be

the case in its sister species, the African *N. constricta* (Kuntner et al. 2008; Su et al. 2011), which shows an unprecedented range of adult female sizes (Higgins et al. 2011).

Biology of the giant wood spider *Nephila pilipes* never ceases to excite, as it is now known to encompass the largest documented mass difference between sexes among terrestrial organisms, with females on average 125 times heavier than males. Furthermore, a previously unstudied developmental mechanism of additional adult growth through molting exists, in addition to a plethora of behavioral adaptations arising through sexual selection that we believe are connected with, if not determined by, extreme SSD (Kuntner et al. 2009a). Notable examples of such behaviors are extreme polygamy, mating plugs, genital mutilation, mate binding (Kuntner et al. 2009b, 2012; Zhang et al. 2011) and the construction of giant asymmetrical webs (Kuntner et al. 2010).

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SHORT COMMUNICATION

Egg sac parasitism of Arctic wolf spiders (Araneae: Lycosidae) from northwestern North America

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Abstract. Parasitoids can have significant impacts on the life history of their hosts, as well as on local population and community dynamics. These effects could be particularly pronounced in the Arctic where the breeding season is short. We studied the incidence and loads of egg sac parasitoids, and whether these varied with body size or among species in three Arctic wolf spider species: *Pardosa sodalis* Holm 1970, *Pardosa lapponica* (Thorell 1872) and *Pardosa moesta* Banks 1892 from the Yukon Territory in northwestern Canada. We found a high incidence of egg sac parasitism (by *Gelis* sp.) and that the incidence of parasitism increased significantly with body size in two of the spider species; however, it did not change in the largest species. Among the three species investigated, parasitism was highest in the largest species (*P. sodalis*). Parasitism loads ranged from one to fourteen individuals per egg sac, and incidence reached 29.6% overall in *P. sodalis*. Parasitism may have significant impacts on the life history of tundra wolf spiders.

Keywords: *Gelis*, *Pardosa*, parasitoid, tundra, Yukon Territory

Parasitoids are efficient predators and can have significant effects on local host population densities (Polis et al. 1998). Parasitoid loads can vary with life history trade-offs and host quality (Fletcher et al. 1994), while their incidence may be influenced by host availability (Le Lan et al. 2012). Wasps belonging to the families Ichneumonidae (e.g., *Gelis* sp.) and Scelionidae (*Baeus* sp.) are known to parasitize the egg sacs of wolf spiders (Edgar 1971; Cobb & Cobb 2004) with the former known to destroy the entire contents of the egg sac (Edgar 1971). Spider densities can be heavily influenced by abiotic factors (Bowden & Buddle 2010), but are also likely affected by local biological factors like predation [i.e., birds and arthropods (Meltofte et al. 2007)], and these could be particularly pronounced in Arctic environments.

Spiders are one of the most speciose arthropod taxa in the Arctic, especially in northwestern North America (e.g., Dondale et al. 1997), with wolf spider species often reaching very high densities (Bowden & Buddle 2012). Female wolf spiders are notable in that they invest unusually heavily in their reproduction. They invest not only in production of the egg sac, but because the egg sac is tethered to them as they move about and they also invest in its incubation and protection. This female movement, however, also likely makes the egg sac more conspicuous to parasitoids and predators. To examine if parasitoids could be important to the ecology of arctic wolf spiders we wanted to determine whether the incidence of parasitoids and parasitoid loads varied among species or with body size within species found on the tundra in the Yukon Territory, Canada.

Sampling was conducted from late June to early August 2008 at three tundra sites in the northern Yukon Territory, Canada: Tombstone, 64.36261N, 138.19411W, elev. 1200m; Ogilvie, 65.47404N, 137.46.206W, elev. 862m and Richardson, 66.55.546N, 136.19.874W, elev. 534m. We selected these three sites to maximize the spatial extent of our study and the collection of our focal species. All three sites were very similar in vegetation composition with representatives of *Ledum* sp., *Vaccinium* sp., *Rubus chamaemorus*, *Cladonia rangiferina* (and other lichens), *Empetrum* sp., *Betula glandulosa* and *Salix* sp. We sampled spiders from late June–early July, mid-July and late July–early August at the Tombstone and Richardson sites and mid-July and late July–early August at the Ogilvie site. Each sampling period consisted of three to four days of collecting in an area of approximately 1 km^2. Our sampling window represents the majority of the active period for arthropods in the region.

We collected live gravid female spiders by visual surveys and with dry pitfall traps at each site. Pitfall traps consisted of 750ml plastic containers dug into the ground with the rim flush to the substrate. They were emptied every five to six hours for the extent of the each sampling period. Our focal species were *Pardosa lapponica* (Thorell 1872), *Pardosa sodalis* Holm 1970 and *Pardosa moesta* Banks 1892, as they represent 46% (28%, 8%, 11%, respectively) of the activity density (abundance) of all spiders in this region (Bowden & Buddle 2010). Gravid female spiders were collected in the field across most of the active season so as to limit bias (detecting many parasitized late in season or few early in season) in the data (Edgar 1971) and subsequently preserved in 70% ethanol. Following gravid spiders through most of the active growing season also allowed us to document the parasitoids' ontogeny to the imago stage. Spider egg sacs are notably darker and more oval when parasitized by wasps like *Gelis* late in the season when larvae have pupated.

All specimens were returned to the laboratory where carapace width of each female was measured to determine body size and the respective egg sac dissected, and contents evaluated. We collected two genera of wasps: *Gelis* and *Baeus*; however, due to the low presence of *Baeus* sp. (eight in *P. moesta*, two in *P. sodalis* and six in *P. lapponica*; all collected from the Ogilvie site) we did not pursue statistical analyses with this genus. Representatives of the adult wasps were sent to the Canadian National Collection of Insects, Arachnids and Nematodes in Ottawa, Canada for identification/confirmation and could only be identified to the genus level; hence it is possible that multiple species occurred. The morphology (e.g., wings) of *Gelis* wasps is known to differ between the sexes (Edgar 1971) and to be influenced by environment and larval host (Salt 1952), making identification to species cumbersome. As the ecology of known species in the genus *Gelis* is similar, however, in having the same ultimate effect upon the spider progeny, our study is relevant to the spiders' ecology. Measurements were conducted using a Nikon® SMZ1000 stereomicroscope fitted with an ocular micrometer. Voucher specimens can be found at McGill's Lyman Entomological Museum, Sainte Anne de Bellevue, Québec, Canada.

Pardosa lapponica is found in high abundance on tundra and less frequently in sparse boreal forest (Bowden & Buddle 2010). *Pardosa*

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Figure 1.—Adult *Gelis* sp. that emerged from an egg sac of *Pardosa* sp. on the tundra in the Yukon Territory, Canada.

sodalis is likely restricted to moist tundra (Dondale & Redner 1990; Bowden & Buddle 2010). *Pardosa moesta* is a generalist species found throughout much of North America (Dondale & Redner 1990; Buddle 2000) and is the smallest wolf spider collected in this region (Bowden & Buddle 2010).

To determine whether the incidence of *Gelis* (Fig. 1) increased with spider size within species we fitted logistic regressions (presence/absence) of *Gelis* to female body size with site as a random factor using generalized linear mixed models (GLMM) with Laplace approximation and log-likelihood ratio tests to test the significance of body size. We examined the proportion of individuals in each spider species parasitized by *Gelis* and determined if larger spiders, within species, hosted more parasitoids using linear regressions. All regressions and ANOVAs were conducted with the base statistics package and GLMMs using lme4 (Bates et al. 2011) for the R environment version 2.15.0 (R Development Core Team 2012).

We collected a total of 574 *P. lapponica*, 253 *P. sodalis* and 121 *P. moesta* and found high incidences of wolf spiders parasitized by *Gelis* on the Yukon tundra (Table 1). We detected significant variation among sites, with the lowest incidence in all three species at the Richardson site. This site variation did not track differences in spider body size (Table 1) and could reflect a context dependency related to some unmeasured biotic (e.g., hyperparasitoids) or abiotic (e.g., temperature) factor(s).

The incidence of *Gelis* parasitoids increased significantly with body size in two of the three species: *P. lapponica* ($X^2_1 = 4.06$, $P = 0.04$) and *P. moesta* ($X^2_1 = 5.33$, $P = 0.02$), but not in *P. sodalis* ($X^2_1 = 1.08$, $P = 0.29$). The highest incidence of *Gelis*, however, was found in the largest species (*P. sodalis*: Table 1). Interestingly, we also found that *P. sodalis* appears to be the first species of the three to emerge from the egg sac (Bowden 2011). We found no significant effect (all $R^2 \leq 0.01$ and P-values $\gg 0.05$) of body size, a proxy for potential clutch size, on parasitoid load for any of the wolf spider species examined.

Table 1.—Incidence of egg sac parasitoids in three *Pardosa* spp. collected from three tundra sites in the Yukon Territory, Canada. The data for *P. moesta* were not available from the Tombstone site. Body size measured as carapace width in millimeters. F-statistics with degrees of freedom are shown above each species tested for body size variation among/between sites. Significant differences are indicated with letters, using Tukey's HSD or ANOVA (for *P. moesta*) at $\alpha = 0.05$.

		Mean body size (± SE)	Percent parasitized
		$F_{2,571} = 119.94$	
P. lapponica	Tombstone	2.175 (± 0.007)a	13.55 (N=214)
	Ogilvie	2.334 (± 0.009)b	43.90 (N=164)
	Richardson	2.309 (± 0.008)c	9.18 (N=196)
		$F_{2,250} = 18.79$	
P. sodalis	Tombstone	2.536 (± 0.012)a	48.54 (N=103)
	Ogilvie	2.641 (± 0.019)b	51.50 (N=33)
	Richardson	2.641 (± 0.014)b	6.84 (N=117)
		$F_{1,119} = 0.14$	
P. moesta	Ogilvie	1.913 (± 0.012)a	5.00 (N=40)
	Richardson	1.920 (± 0.019)a	1.23 (N=81)

To our knowledge, the percentages of parasitized egg sacs we report are the highest on record and are almost 1.5 times greater than the highest recorded in other populations of wolf spiders (Eason et al. 1967; Edgar 1971; Cobb & Cobb 2004). Edgar (1971) found total egg sac parasitism, by *Gelis* and *Hidryta*, to range from 2.9% to 34.8% of individuals in *Pardosa lugubris* (Walckenaer) in Scotland. The high overall incidence of parasitism by *Gelis* in the region of our study may reflect high resource availability of prey (i.e., spiders) with little refuge. Therefore, high rates of parasitism could also be present in other open habitats (e.g., alpine tundra, coastal barrens) where wolf spiders are abundant.

Our findings suggest that these parasitoids could have important consequences for the reproductive fitness of these northern spider species since *Gelis* destroy the contents of the egg sac, rendering the female's fitness effectively equal to zero (Edgar 1971; pers. obs.). This idea is supported by our data because we collected gravid female spiders throughout most of the active season (active season being late May to late August), and our sampling shows that it takes an entire season to mate, produce the clutch and carry it until the progeny hatch late in the summer (Bowden 2011). Like high elevation species (Schmoller 1970), our study species probably take multiple years to reach maturity, and this is supported by the overlapping generations we collected in pit-fall traps. Hence, in our study area we suspect that females produce just one egg sac per lifetime as it is unlikely that females overwinter to produce subsequent clutches in following seasons (Kiss & Samu 2002).

The fact that we found the highest incidence of *Gelis* parasitoids in egg sacs of *P. sodalis* and the lowest in *P. moesta* suggests that targeting the largest spider species may maximize the parasitoids' fitness. This would make sense from the perspective of the wasp, as it could maximize resources available for the wasp's progeny; however, we did not find that body size (within spider species) significantly predicted parasitoid loads in egg sacs. Perhaps variation in actual clutch size (e.g., larger spiders producing smaller clutches) skews this relation or there is a balance to be struck between host clutch mass and number (Pérez-Contreras & Soler 2004) and its own mass. We did find, at least for *P. moesta* and *P. lapponica*, that the probability of being parasitized increases with body size, and this may reflect increased movement by larger individuals or parasitoids able to assess this trait. We did not find the same with *P. sodalis*, so it is possible that a threshold is reached for the parasitoid where it does not pay or is not possible to produce and lay more eggs in larger spider egg sacs.

Further experiments would have to be employed to fully test these ideas.

Our study shows that egg sac parasitoids are frequent in populations of Arctic wolf spiders from the tundra in the Yukon Territory, Canada. Evidence exists in other high Arctic arthropod groups that parasitoids may have an important influence on the life history of host speeies (Kukal & Kevan 1987). Although little is known about the parasitoids themselves, we have shown that their phenology seems to track that of the spiders quite well; however, it could vary from year to year. Hence, this study reveals many new questions in the contexts of community, population and behavioral ecology. Understanding more about the parasitoids and their impacts on local population and community dynamics, both spatially and temporally, would be particularly interesting.

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CONTENTS

Journal of Arachnology

Volume 40 Number 3

Featured Articles

Distribution and morphology of the European Karst palpigrade *Eukoenenia gasparoi* (Arachnida: Palpigradi)
by Erhard Christian, Ľubomír Kováč, Roman Ozimec, Slavko Polak & Maja Zagmajster 265

A new cave-dwelling species of *Spelaeobochica* (Pseudoscorpiones: Bochicidae) from Brazil
by Pedro Ratton, Volker Mahnert & Rodrigo Lopes Ferreira 274

Multivariate methods support the distinction of a new highland *Vaejovis* (Scorpiones: Vaejovidae) from the Sierra de los Ajos, Mexico **by Matthew R. Graham, Richard F. Ayrey & Robert W. Bryson, Jr.** 281

A new species of *Heterolacurbs* (Opiliones: Biantidae: Stenostygninae) from Puerto Rico
by Aylin Alegre Barroso & Luis F. de Armas 291

The identity of *Hadrobunus grandis*: reassignment of *Leiobunum aurugineum* to *H. grandis* and *H. nonsacculatus* new species (Opiliones: Sclerosomatidae: Leiobuninae) **by Jeffrey W. Shultz** 296

Comparative study of walking and climbing speeds among Neotropical harvestmen from Costa Rica
by Adam T. Smith, Dayna R. Cook, Megan B. Johnson & Victor R. Townsend, Jr. 304

Epigeal spider responses to fertilization and plant litter: testing biodiversity theory at the ground level
by L. Brian Patrick, Mark W. Kershner & Lauchlan H. Fraser 309

Notes on the ecology and behavior of a subsocial spider *Anelosimus baeza* (Araneae: Theridiidae) in Mexico
by Dinesh Rao & Alfonso Aceves-Aparicio 325

Behavioral analysis of the interaction between the spitting spider *Scytodes globula* (Araneae: Scytodidae) and the harvestman *Discocyrtus invalidus* (Opiliones: Gonyleptidae)
by Luanda Abrão Carvalho, Elene da Silva Souza & Rodrigo H. Willemart 332

Static allometry and sexual size dimorphism in *Centruroides margaritatus* (Scorpiones: Buthidae)
by Catalina Sánchez-Quirós, Edgardo Arévalo & Gilbert Barrantes 338

Short Communications

Nephila female gigantism attained through post-maturity molting
by Matjaž Kuntner, Shichang Zhang, Matjaž Gregorič & Daiqin Li 345

Egg sac parasitism of Arctic wolf spiders (Araneae: Lycosidae) from northwestern North America
by J. J. Bowden & C. M. Buddle 348

Instructions to Authors 351